PRECLINICAL SAFETY EVALUATION OF AYAPODI ELAGAM

The Dissertation Submitted by

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Dissertation Submitted to

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY,

Chennai – 32.

For the partial fulfillment of Requirements to the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH VI - DEPARMENT OF NANJU NOOLUM

MARUTHUVA NEETHI NOOLUM

2015 – 2018

NATIONAL INSTITUTE OF SIDDHA

Chennai – 47.

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Preclinical safety evaluation of AYPODI ELAGAM"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.P.Shanmugapriya, M.D(S).,** Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Chennai -47, and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or another similar title.

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BONAFIDE CERTIFICATE

Certified that I have gone through the dissertation submitted by **Dr.A.Kalaivani (Reg.No: 321516203)** a student of final year M.D(S), **Branch-VI, Department of Nanju Noolum Maruthuva Neethi Noolum**, National Institute of Siddha, Tambaram Sanatorium, Chennai - 47, and the dissertation work has been carried out by the individual only. This dissertation does not represent or reproduce the dissertation submitted and approved earlier.

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ACKNOWLEDGEMENT

- I thank God for giving me this opportunity, providing the strength and energy to fulfill this commitment.

- I express my sincere thanks to the Secretary, Ministry of AYUSH, Health &Family Welfare, New Delhi for giving great opportunity to carry out P.G and this dissertation work in National Institute of Siddha, Chennai-47

- I express my sincere thanks to the Vice-chancellor, The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

- I express my profound sense of gratitude to Prof. Dr.V.Banumathi, M.D(S), Director, National Institute of Siddha, Chennai-47 for granting permission to undertake a study in this dissertation topic and also for providing all the basic facilities in order to carry out this work.

- I express my sincere thanks to Dr.R.Madhavan M.D(S), Lecturer, Head of the Department. i/c, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Chennai-47 for the guidance, memorable support and ceaseless encouragement in carrying out this work.

- Dr. P. Shanmugapriya M.D(S), Lecturer and my Guide, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Chennai -47. She not only teach us but also extended all valuable help and advice during the course of this study, without her help this work could not have seen the light of the day.

- I would like to express my sincere thanks to my Dr. R.Renga Sundari, M.D(S), Associate Professor, Dept. of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Chennai-47 for her encouragement in carrying out this work.

- I express my grateful thanks to Dr. V.Manjari, M.D(S), Lecturer, Dept. of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Chennai-47 for the guidance and encouragement in carrying out this work.

- I express my sincere thanks to Dr. S.Murugesan, M.D(S) Lecturer, Dept. of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha for the guidance and encouragement in carrying out this work.

- I am thankful to Dr.D.Aravind MD(S) Assistant Professor, Dept. Of Botany, National Institute of Siddha, chennai-47, and Dr.S.Visweswaran, M.D.(S) Lecturer, Dept.of. Gunapadam, National Institute of Siddha, chennai-47 to help me to authenticated my drugs.

- I thank Dr. A. Muthuvel, M.Sc., Ph.D. (Biochemistry) Assistant Professor, National Institute of Siddha, Chennai-47 for his guidance in doing chemical studies.

- My special acknowledgments to Mr. M. Subramanian, M.Sc., (Statistics), Senior Research Officer, National Institute of Siddha, Chennai-47, for his valuable help in statistical analysis.

- I thank Dr. V. Suba, M.Pharm., Ph.D., Assistant Professor, Dept. ofPharmacology, National Institute of Siddha, Chennai-47 for her interesting teaching of pharmacology and valuable guidance for doing this study.

- I thank Dr.Jeyanthinath, Asst. Prof, Department of Materials Science, M.K.University, Madurai-625021 for his valuable help in Spectroscopic Analysis .

- I thank Dr.V.Shanmugaiah, Asst.Prof, School of Biological Sciences and Mr.Suresh, Ph.D Scholar, School of Biological Sciences , M.K.University, Madurai- 625021 for his help in Microbial activity studies.

- I thank the library clerk Mrs. V. Kalpana, Mr.J.Rathinam library attendant of National Institute of Siddha, Tambaram Sanatorium, Chennai-47, from where I derived much of the literary support.

- I thank the Dr. Gayathri, B.V.Sc.National Institute of Siddha, Tambaram Sanatorium, Chennai-47 for her valuable guidance for doing this study.

- I gratefully acknowledge the assistance provided by all other faculties, Well-wisher and staffs of NIS, Chennai who rendered their cooperation throughout the course of study.

- I express my sincere thanks to Dr.D.Sivaraman Scientist-Department of Pharmacology and Toxicology, Centre for Laboratory Animal Technology and Research, Dr. Jeppiaar Research Park, Sathyabama University Chennai for the guidance of histopathology study in this work.

- I thank all my dear friends, seniors and juniors who helped me throughout the study, without whom this work will be impossible.

- I wish to thank the Animal house attenders for doing various helps in animal house.

- I wish to thank the students GHSS, Sirupakkam who helped me to collected the raw drug for this study.

- I wish to dedicate this work to my dear parents, brother, sister and uncle who are helping and sacrificed everything for me and they support in every stage of my work and life.

- I remind thankfully all the animals that lost their lives for the sake of my study and without which I would not have been successful in my study.

CONTENT

CHAPTER I

INTRODUCTION

Siddha is one of the ancient medical systems in India considered as the mother medicine of ancient Dravidians in South India. The word Siddha means established truth or "one who is accomplished".

The term ‘Siddhar’ has derived from the word ‘siddhi’ which literally, means accomplished, achieved, or perfected success; and so it refers to one who had attained his end in spiritual goal. The word ‘Siddha’ does not refer to those men who have become free- a sense in which it is often used.

The Siddhar’s had investigated and studied the cause and effect of disease and all kinds of drugs, mineral and poisons and thereby came to realize what is beneficial and what was not to their existence in life. They can even, if they choose retain their bodies for ages of disintegrate them at their will and can also dematerialize or rematerialize their bodies as they liked.[1]

In Siddha medicine the use of metals and minerals are more predominant in comparison to other Indian traditional medicine systems.The drugs used by the Siddhar’s could be classified into three groups: Mooligai/Thavaram (herbal product), Thathu (inorganic substances) and Jeevam or Sangamam (animal products). The Thathu drugs are further classified as:

1. Loham (Heavy metals)
2. Paashanam (Arsenicals)
3. Uppu (Salts)
4. Rasam (Mercury and its salts)

The use of more metals and chemicals was justified by the fact that to preserve the body from decomposing materials and it has more potency to treat the chronic diseases. One among the metal is Ayam which is commonly used by Siddha practitioners for treating various diseases.

According to their mode of application the Siddha medicine could be categorized into two classes:

1. Internal medicine and
2. External medicine.

- Internal medicines are used through the oral route and further classified into 32 categories based on their form, method of preparation, shelf life, etc.
- External medicine includes certain form of drugs and also certain applications like nasal, eye and ear drops and also certain procedures like leech application.

The internal medicines are 32 in number. They include medicines with short life period to medicines which could be used even for hundreds of years. The metallic preparations that could be preserved and used for longer duration are considered as higher medicines. They act even in very small quantity and are capable of curing chronic illness. Elagam was one among them.

In Siddha literature Elagam maens some decoctions or juices are mixed together, sugar is added to it and boiled in the mild fire. When an aromatic smell appears, fine powders are sprinkled into it. Ghee is added to it and then, honey is added until it is mixed well.[2]

Ayapodi Elagam is one among the elagam preparation in Siddha system of medicine. The ingredient of the drug was Ayam, Nellikai, Keezhanellai and Karisalai. It is used for treating anaemia, jaundice etc.It impoves and purifies the blood. Iron is the most important metal used in Siddha system of medicine for treating pandu. The Siddha medicines meant for the human body are prepared, based on the theory of Panchabootham.The metals also calcified based on panchabootham theory.

- Gold–Earth
- Lead – Water
- Copper – Fire
- Iron – Air
- Zinc –Space

Gold and lead are used for the maintenance of the body. Iron, the only metal attracted by the electric power of the magnet, and zinc used for generating electricity are employed in

the medicines which are administered for the extension of life and copper is used for the preservation of heat in the body.

The external five elements correspond to the internal Vatham, Pitham, and Kaphamof the human body. This concept is described by the great sage Sattamuni as,

அண்டத்தில் உள்ளதோ பிண்டம்
பிண்டத்தில் உள்ளதோ அண்டம்.

The treatments for the imbalance of the three humors are made up of the five elements. The drugs are made up of the five elements. By substituting a drug of the same constituents (gunam) the equilibrium is restored. The correction of the imbalance is made by substituting the drug which is predominate of the opposite nature.[3]

Pandu is a pitham related disease.So the medicine which is used for the treatment of pandu that medicine has to balance the deranged pitham in our body[4].

Anaemia is the most prevalent nutritional deficiency disorder in the world. It is a condition that occurs when the red blood cells do not carry enough oxygen to the tissues of the body.

According to World Health Organization criteria, anemia is defined as blood hemoglobin (Hb) concentration <130 g/L (<13 g/dL) or hematocrit (Hct) <39% in adult males; Hb <120 g/L (<12 g/dL) or Hct<37% in adult females.

A physiologic approach to anaemia diagnosis is based on the understanding that a decrease in circulating RBCs can be related to either inadequate production of RBCs or increased RBC destruction or loss. Within the category of inadequate production, erythropoiesis can be either ineffective, due to an erythrocyte maturation defect, or hypoproliferative.[5]

Most of the anemias are due to an inadequate supply of nutrients like iron, folic acid and vitamin B12, proteins, amino acids, vitamins A, C, and other vitamins of B-complex group i.e., niacin and pantothenic acid are also involved in the maintenance of haemoglobin level.

Globally, anaemia affects 1.62 billion people, which corresponds to 24.8% of the population. The highest prevalence is in preschool-age children (47.4%), and the lowest prevalence is in men (12.7%).

Prevalence of anaemia in all the groups is higher in India as compared to other developing countries.In India anaemia affects an estimated 50% of the population. The problem becomes more severe as more women are affected by it as compared to men.[6]

According to Siddha literature lot of Ayam containing preparations are mentioned for treating pandu. One of the medicine is Ayapodi Elagam. In this medicine not only in Ayam the ingredients like Karisalai, Nellikai and Keezhanelli also used for treating pandu.It is used by many Siddha practitioners,yet this safety is not proven.

So I proposed to take Ayapodi Elagam for my dissertation study to evaluate the safety profile on animal model.

CHAPTER II

AIM AND OBJECTIVES

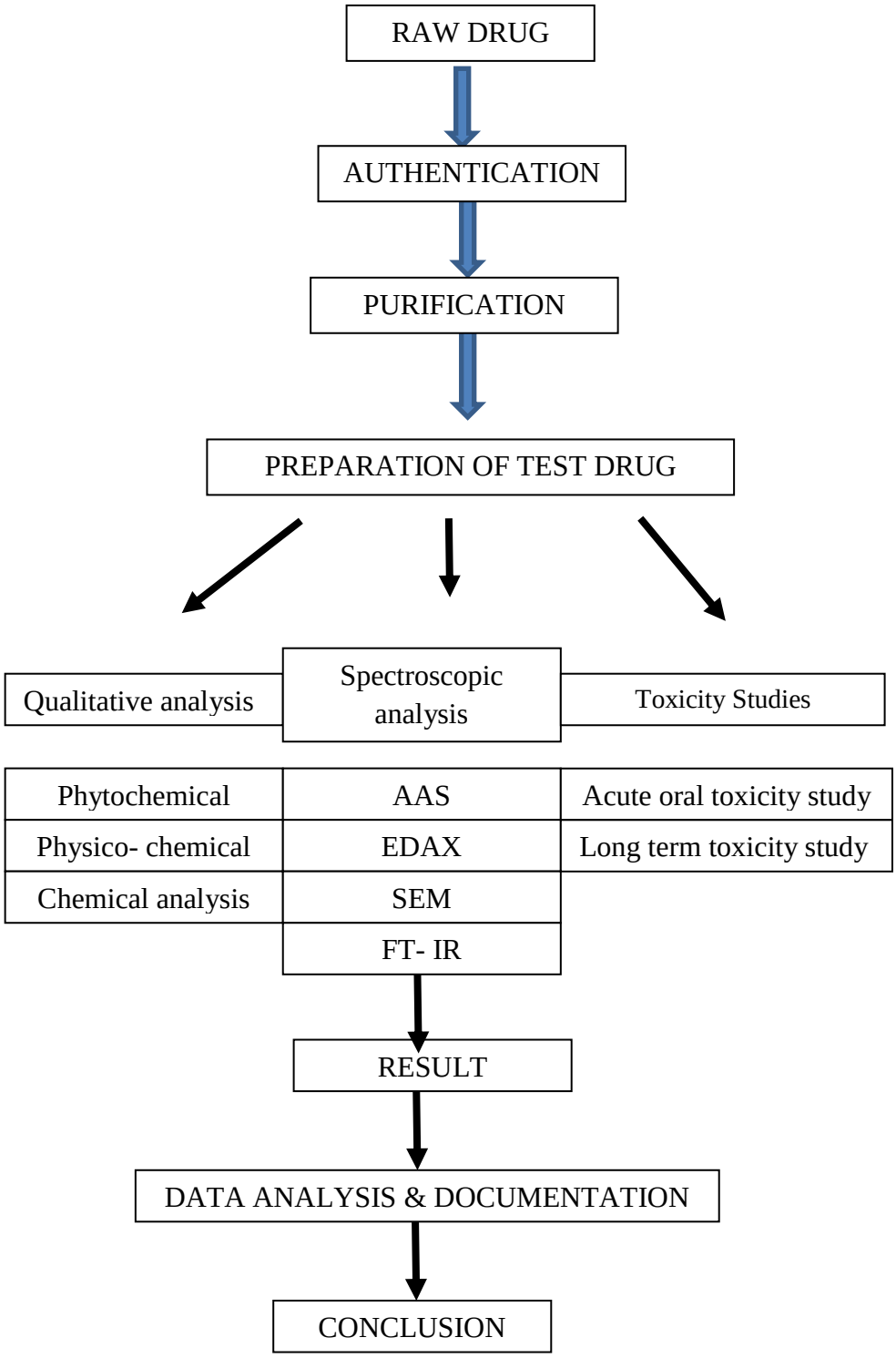
AIM:

To evaluate the safety profile of Ayapodi Elagam (A.E) on animal model (wistaralbino rats).

OBJECTIVES:

- To study the physicochemical properties, biochemical analysis and phytochemical analysis of Ayapodi Elagam(A.E).
- To study the spectroscopic analysis of Ayapodi Elagam(A.E).
- To evaluate the acute toxicity profile of Ayapodi Elagam as per WHO Guideline.
- To evaluate the long term toxicity profile of Ayapodi Elagam as per WHO Guideline.

WORK PLAN


RAW DRUG
AUTHENTICATION
PURIFICATION
PREPARATION OF TEST DRUG
Qualitative analysis
Spectroscopic analysis
Toxicity Studies
Phytochemical
Physico- chemical
Chemical analysis
AAS
EDAX
SEM
FT- IR
Acute oral toxicity study
Long term toxicity study
RESULT
DATA ANALYSIS & DOCUMENTATION
CONCLUSION

CHAPTER III

அயம்

வேறுபெயர்:

அகி, அயசு, அயில், இடி, இரும்பு, ஈசசெயம், கருங்கொல், கருப்பி, கரும்பு, கருப்பு, கருமணல், கரும்பொன், கயசு,கிருஷ்ணவையம், காலில் நெகிளம், லோகம், சிட்டம், பொன்மணல், சத்து, வாழ்பூமி நாதம்,பிண்டம், கருந்தாது.

பகைசரக்கு:

- **அப்பிரகம்.**
- **அண்டவோடு.**
- **கந்தி.**
- **கௌரி.**
- **சவ்வீரம்.**
- **சாரம்.**
- **சிங்கி.**
- **சிலாசத்து.**
- **சிலை.தரா.**
- **வெங்காரம்.**
- **வெள்ளைப்பாடாணம்.**

நட்புசரக்கு:

- **காந்தம்.**
- **சூடன்.**
- **செம்பு.**
- **தங்கம்.**
- **நாகம்.**
- **பூரம்.**
- **வெள்ளி.**
- **பூநாகம்.**
- **மயூரச்செம்பு**

சுவை : துவர்ப்பு. சிறு புளிப்பு.கைப்பு

தன்மை: வெப்பம்

செய்கை:

பசியுண்டாக்கி
உடல் உரமாக்கி
குருதிபெருக்கி
உடல்தேற்றி

குணம் :

“பாண்டுவெண் குட்டம் பருந்தூல நோய்சோபை
மாண்டிடச்செய் மந்தங்கா மாலைகுன்மம் பூண்ட
பெருந்தாது நட்டமும்போம் பேதிபசி யுண்டாங்
கருந்தாது நட்டமிடுங் கால்”.

இரும்பினால் பித்தப்பாண்டு, வெண் குட்டம், அதிதூலநோய், சோபை, மந்தம், காமாலை, குன்மம், சுக்கிலநட்டம், கழிச்சல்,இவை நீங்கும்,பசி உண்டாகும்.

பழமொழிகள்:

“இளைத்தவர் இரும்பை உண்ண வேண்டும்”
“எய்ப்புடற் கிரும்பை யுண்மின்”

சுத்திமுறைகள்:

1. **ஒரு பலம் அயப்பொடிக்கு ஆறு பலம் இலுப்பைப் பூச்சாறு விட்டு. காலை முதல் மாலை வரை வெயிலில் வைக்க வேண்டும். இவ்விதம் ஆறு நாள் செய்து இரண்டு நாள் சாறு விடாமல் உலர்த்தி , பின்னும் இதைப் போல இருமுறை செய்து, 25 ஆம் நாள் முதல் 10 நாட்கள் இடைவிடாமல் மேற்படி சாறுவிட்டு, வெய்யிலில் வைத்துப் பின்பு, சாறுவிடாமல் இரண்டு நாள் உலர்த்தி நீர்விட்டுக் கழுவி எடுக்கச் சுத்தியாம்.**

2. **அயத்தைக் கொல்லன் உலையிலிட்டுச் சிவக்க காய்ச்சி ,ஆறுமாத அன்னக்காடி, எண்ணெய், ஆவின்நீர், கொள்குடிநீர் இந்நான்கிலும் முறையே மும்மூன்று முறை தோய்த்துத் தோய்த்தெடுத்துக் கழுவிக் கொள்ளச் சுத்தியாம்.**

3. **இரும்பின் அரப்பொடியை எலுமிச்சை பழச்சாறு, காடி, நாட்டுக் காட்டாமணக்குப் பால் இவை ஒவ்வொன்றிலும் மூன்று நாள் ஊற வைத்துக் கழுவியெடுக்கச் சுத்தியாம்.**

4. **அயப்பொடிக்கு நாவற்பழச் சாற்றை மூழ்கும்படி விட்டு சாறு சுண்டும் வரை வெயிலில் வைத்துக் கழுவுக. இவ்விதம் ஆறுமுறை செய்ய அயம் சுத்தியாம்.**

5. இரும்புப்பொடியை ஒரு நாள் பழரசத்தில் ஊறவைத்து எடுத்து மறுநாள் எண்ணெயில் ஊறவைத்து அயச்சட்டியில் போட்டு வறுத்து காடியில் கழுவி வேலம்பட்டை கியாழத்தில் கொதிப்பித்துதிரும்பவும் காடியில் கழுவி எடுத்துக்கொள்ளவும்.

தோடம் :

அயத்திற்கு ஐந்தோடங்கள் உண்டு.

"வாறுகே ளிரும்புக்கைங் குணங்க ளுண்டு
வகையாகச் சொல்லுகிறேன் நன்றாய்க் கேளும்
தாறுகேள் திரைசவிடு வுடைச்ச லூறல்
சரசமா யிருக்காத குணந்தா னைந்தும்
பேறுகே ளிதைநீக்க வறிந்தோன் வாதி
பிரித்திதனை நீக்காதான் பிணந்தான் பாரே
வாறுகேள் குளம்வெட்ட வூற்றுப் போல
வம்மம்மா விரும்பூறல் அருகா தென்னே."

சுத்தி செய்யப்பட்ட அயத்தைப் பற்பம், செந்தூரம், களங்கு, மெழுகு,வடகம், பாணிதம் முதலிய மருந்துகளாக்கி உபயோகித்தல் வேண்டும்.

இவற்றுள், பற்பம் - முழுவன்மையும்
செந்தூரம் - முக்கால் வன்மையும்
மற்ற மருந்துகள் - அதற்கும் குறைந்த வன்மையும்கொடுக்கும்.[2]

அயம் சேரும் பிற மருந்துகள்:

1.அயச்செந்தூரம் [8]
அளவு : துவரையளவு
அனுபானம் : தகுந்தஅனுபானம்
தீரும்நோய் : தாதுவிருத்தி, நல்லதேஜசம் உண்டாகும்

2.அயகாந்த செந்தூரம்[9]

அளவு : ½ முதல் 1 குன்றி(65-130mg)
தீரும்நோய் : பாண்டு, சோபை,சுரம்

3.அயகாந்தசண்டமாருதச் செந்தூரம்

அளவு : ½ முதல் 1 குன்றி(65-130mg)
தீரும்நோய் : பாண்டு, சோபை,காமாலை

4.வீரஅயச்செந்தூரம்

அளவு : ½ முதல் 1 குன்றி(65-130mg)
அனுபானம் : நெய்,தேன், வெண்ணெய்
தீரும்நோய் : பாண்டு, சோபை,காமாலை

5.நீலகண்ட செந்தூரம்

அளவு : 2 அரிசி
அனுபானம் : நெய், தேன்
தீரும்நோய் : பாரிசவாதம், முகவாதம், ஜிக்குவாதம்

6.வங்கச் சந்திரோதயம்

அளவு : 1\4 முதல் 1\2 குன்றி(32.5-65mg)
அனுபானம் : திரிகடுகு சூரணம்
தீரும்நோய் : பித்தம், வாயு, அக்கினிமந்தம், காமாலை, சோபை

7.சத்து செந்தூரம்

அளவு : 1\4 குன்றி(32.5mg)
அனுபானம் : நெய், தேன்
தீரும்நோய் : நரம்பின் துர்பலம், இரத்தகுறைவு, மதுமேகம்

8.அயச்சூரணம்[10]

அளவு : குன்றியெடை(130mg)
அனுபானம் : நெய், தேன்
தீரும்நோய் : எல்லாவகைபாண்டு, கிராணிகள்

9.சுயமாக்கினி

அளவு : 2-3 குன்றி(260-390mg)
அனுபானம் : நெய், தேன், இஞ்சி இளகம்
தீரும்நோய் : குன்மம், பெருங்கழிச்சல், அக்கினிமந்தம், சுவையின்மை

10. லோக செந்தூரம்

அளவு : 2-3 குன்றி(260-390mg)
அனுபானம் : நெய், தேன்
தீரும்நோய் : பித்தவெட்டை, மஞ்சள்நோய், நச்சுபேதி, அசிரணம்

11.வருணலோக செந்தூரம்

அளவு : 1\4 முதல் 11\2 குன்றி(32.5-195mg)
அனுபானம் : தேன், நெய்
தீரும்நோய் : பாண்டு, காமாலை

12.சுண்ணலோக செந்தூரம்

அளவு : 1 1\2 - 3 குன்றி(195-390mg)
அனுபானம் : தேன், நெய், வெள்ளரிவிதை
தீரும்நோய் : பாண்டு, பீலிகை, பெருவயிறு

13. எ.ுகுசுவர்ணசெந்தூரம்

அளவு : 1 - 2 அரிசி
அனுபானம் : தேன், பாலேடு,கோதுமைபால்
தீரும்நோய் : பாண்டு, அசதி, சயம், மூளைநலக்குறைவு, இரத்தங்கக்கல்[10]

14. அயத்தங்கச் செந்தூரம்[9]

அளவு : 1\2 முதல் 1 குன்றி(65-130mg)
அனுபானம் : தேன், நெய்
தீரும்நோய் : காசம், பாரிசவாதம்,முகவாதம்,காமாலை, மதுமேகம்

15. அயவெள்ளிச் செந்தூரம்

அளவு : துவரையளவு
அனுபானம் : தாதுவிருத்திலேகியம்
தீரும்நோய் : நாட்பட்டதேகதுர்பலம்,தாதுநட்டம்

16. சின்னபட்டுக்கறுப்பு

அளவு : துவரையளவு
அனுபானம் : தேன்
தீரும்நோய் : மேகசுரம்,யானைகால்,சுரம்,வயிற்றுவலி.

AYAM (FERRUM)

Eng : Iron

Sans : Lauha

Tam : Irimbu

Tel : Inumu

Hind : Loha

Urdu : Lohchum

Source:

Rarely met with free in nature, though very widely distributed in both organic and inorganic kingdom.Founds nearly all rocks, soils, etc., variously combined with oxygen as haematite, magnetic iron ore etc., with sulphur as iron- pyrites, and as a carbonate of iron, in spathi iron; in the ashes of plants and even the blood of animals; also in the bile, chyle, gastric juice, lymph, milk, pigment of the eye and in the urine.[11]

Iron ores:

Next to the aluminium iron is the most abundant metallic element comprising about 4.44% of the earth crust. It does not occur in the metallic form except rarely in meteorites. It is found mainly in the form of oxides hydrated oxides, carbonates, sulphides, silicate and hydrated oxides of iron and aluminium. Chromites and ilmenite are iron-bearing minerals.Iron is sometimes obtained as a byproduct in the working of pyrites and ilmenite.

Origin and mode of occurrence:

Only a few of the world's iron ore deposits are of igneous origin .The great majority of them have been formed by deposition from sulphur or underground waters. The iron being normally derived by solution from preexisting rocks under ordinary temperature conditions.In some cases the iron minerals have been concentrated in the form of iron sands. Electrolytes occurring in natural waters may also precipitate iron.

Compared most producing countries the quality of iron ores mined in India is superior. India iron ore deposits are among the finest and largest in the world the average ore worked at present containing over 60% iron.

In Tamilnadu:

Iron ore deposits of some importance occur in Salem and Tiruchirapalli districts. Several occurrences in other districts are known but they are not considered important at present.

Salem and Tiruchirapalli extensive deposits of magnetite quartz rocks occur in a series of ridges and hillocks in these districts. The major deposits are kanjamalai, godumalai, perunamalai, pachamalai, kollimalai and chitteri.Deposits are also found in several hills 5 miles west south-west of the Salem town is the most important the average iron content is 35- 40%. Grade % of iron in Madras 67 and above.[12]

Physical properties:

Iron is a silvery white or greyish metal. It is ductile and malleable. Ductile means capable of being drawn into thin wires. Malleable means capable of being hammered into thin steels. It is one of only three naturally occurring magnetic elements. The other two are nickel and cobalt.

Chemical properties:

Iron is a very active metal. It readily combines with oxygen in the moist air. The product of this reaction iron oxide is known as rust. Iron also reacts with very hot water and steam to produce hydrogen gas. It also dissolves in most acids and reacts with many other elements.Iron burns with a gold colour in a flame test.[13]

In Siddha aspect:

Iron is found in mountains and in the earth in association with certain material like sulphur. It is also found in some plants and animals.

Iron has astringent and mild sour and bitter taste. It has tonic, haemopoietic, appetite, stimulant and health-promoting properties. It potency is hot.

It improves the properties and functions of blood. Some preparations of iron may cause constipation. In order to prevent it the myrobalans are added to iron preparations.

Iron stimulates the functions of all the organs of the body and thus it acts as a tonic.

Iron preparations are used in treating the disease like anaemia, jaundice, leucoderma, obesity, dropsy, anorexia, peptic ulcer, spermatorrhoea, diarrhoea and dyspepsia.[14]

Iron in modern aspect:

Iron is an essential nutrient utilized in almost every aspect of cell function and its availability has previously limited life. Those same properties which allow iron to function as a catalyst in the reactions of life also present a threat via generation of oxygen-based free radicals. Accordingly life exists at the interference of iron deficiency and iron sufficiency. Iron is an important "blood building mineral".Blood protein such as haemoglobin transport iron in RBC. Hb is critical for iron, oxygen and CO_2 transport. Iron in it's critical for the formation of catalyzing a key antioxidant that protects cells from free radicals by degrading hydrogen peroxide to water and O_2. Inadequate iron means inadequate Hb means low TPO bonding which leads to anaemia.[15]

Recommended Daily intake:

Adult man	:	10 mg/day
Menstruating woman	:	18 mg/day
Pregnant and lactating woman:		40 mg/day[16]

Food:

Iron occurs as a natural constituent in plants and animals. Liver, kidney, fish, and green vegetables contain 20–150 mg/kg whereas red meats and egg yolks contain 10–20 mg/kg. Rice and many fruits and vegetables have low iron contents (1–10 mg/kg). Estimated total exposureand relative contribution of drinking water. Reported daily intakes of iron in food - the major source of exposure range from 10 to 14 mg. Drinking water containing 0.3 mg/litre would contribute about 0.6 mg to the daily intake. Intake of iron from the air is about 25 μg/day in urban areas.[17]

Uses:

- Iron is an essential mineral for health, but too much iron is extremely toxic. Free iron in the blood reacts with peroxides to form free radicals that damage DNA, protein, lipids and other cellular components, leading to illness and sometimes death.

- 20 milligrams of iron per kilogram of body weight is toxic, while 60 milligrams per kilogram is lethal.

- Iron is essential for the development of brain in children. Children with iron deficiencies show a lower ability to learn.[13]

- Iron improves the quality of blood.

- Iron stimulates the functional activity of all the organs of the body and is therefore a valuable general tonic.

- As a haematinic tonic prepared iron is used in many diseases like anaemia and chlorosis.

- Iron has a great value in both simple and secondary anaemias.

- In haemorrhagic diseases such as haemoptysis, haematuria, bleeding from piles etc.Iron is commonly given with good result.

- In leucorrhoea leading to anaemia preparations containing iron are useful.

- Iron oxides and hydroxides are used as pigments in cosmetics.

- Potassium ferricyanide (red prussiates of potash) is obtained from ferrous ferricyanide (Turnbull's blue) and is used in processing blueprint paper.

- The use of iron cooking utensils is often considered a useful source of supplementary iron in the diet.[18]

- Iron is of great value when given internally in some skin diseases i.e., erysipelas, carbuncles and furunculosis.

- In anaemia and dyspepsia with anorexia an organic compound of iron called kalpam made of iron powder, pepper, garlic and limes was tried and "found very beneficial in improving the blood strengthening the patient and also in creating an appetite".[11]

Heavy metal toxicity:

Symptoms of excess:

Anger, liver disease, cancer, iron deposits in organs, diabetes, arthritis, cirrhosis of the liver, schizophrenia, emotional problems, high blood pressure, myasthenia gravis, hemochromatosis and hemosiderosis.

Additional Iron Factors: Excess iron can lead to aggressive behaviour.[19]

Scientific validation:

- This article reveals the iron increase the haemoglobin leavel.The dose of iron which produces an average increase of at least 1% of haemoglobin per day in a substantially large group of patients with achlorhydria and anaemia. To achieve this one must consume at least 25mg of iron.[20]
- Intravenous Iron therapy is effective in increasing the Hb concentration and reducing the risk of allogeneic red cell transfusion and could have broad applicability to a range of acute care settings. This potential benefit is counterbalanced by a potential increased risk of infection.[21]
- This article showed the toxic effect of the iron.Oral administration of Arumuga Chenduram did not induce any toxic effect at 24mg/kg/day dose in rats and this stands as an assurance of safety usage at its desirable human intended therapeutic dosage of 260mg/70kg/day in the practice of Siddha medicine.[22]
- This article reveals the effectiveness of iron preparation.The Ayurvedic iron preparation Sahastrapatti lauha bhasma and Satiputti lauha bhasma may be considered to be better drugs that the allopathic iron preparation ferrous sulphate on the basis of the bioavailability resulting due to increase in the haemoglobin content.[23]
- This article reveals the safety of the iron containing medicine. Lauha Bhasma and Mandura Bhasma in 55mg/kg dose for 60 days exhibited no serious toxic effects in Charles Foster Albino rats. Both the drugs showed significant recovery from chronic toxic effect after 45 days of the recovery period.[24]
- The Acute LD of $FeCl_3$ for sheep therefore seems to be between 2.0- 2.5g/kg while 500mg/kg/day caused death within 3 weeks. A Dosage of 100mg/kg/day over 182 days caused pronounced symptoms and marked accumulation of Fe in soft tissues. As little as 34 mg $FeCl_3$/kg/day therefore causedas accumulation of Fe in soft tissues. It is therefore possible that even low dosages given over long periods might not be without danger.[25]

- This article showed the effect of iron containing medicine against the bacteria. Chandraprabhavati showed a significant reduction in signs and symptoms of Albuminuria mainly albumin in urine and although turbidity, organism, pus cells, red cells colour and frequency of urine as well as the same premonitory symptoms of prameha.[26]
- This article reveals the cardio productive effect of iron.The Cardio productive activity of Fe_2O_3 NPs requires the integrity of Nanoparticles and is not dependent upon their surface changes and molecules that were integrated into nanoparticles. Also Fe_2O_3 NPs showed no significant toxicity towards normal cardiomyocytes, indicative of their potential to treat cardiovascular disease.[27]

3.2 நெல்லிக்காய்

வேறுபெயர்: ஆமலகம்,

ஆலகம்,

ஆம்பல்,

ஆமரிகம்,

தாத்தாரி,

தாத்திரி,

கோரங்கம்,

மிறுதுபலா,

மீதுந்து.

பயன்படும் உறுப்பு: இலை, பூ, பட்டை,வேர்,காய், விதை

சுவை : புளிப்பு, துவர்ப்பு, இனிப்பு

தன்மை : தட்பம்

பிரிவு : இனிப்பு

செய்கை:

துவர்ப்பி	-	Astringent
சிறுநீர்ப்பெருக்கி	-	Diuretic
குளிர்ச்சியுண்டாக்கி	-	Refrigerant
மலமிளக்கி	-	Laxative

குணம்:

"பித்தமன லையம் பீநசம்வாய் நீர் வாந்தி
மத்தமலக் காடும் மயக்கமுமில் - ஒத்துவுரு
வில்லிக்கா யம்மருங்கா மென்னாட்கா லந்தேர்ந்தே
நெல்லிகா யம்மருந் துணீ".

நெல்லிக்காயைப் பகற்பொழுதுண்ணில் வெறி, ஐயநோய்,பீனிசம், வாய்நீர் சுரப்பு,வாந்தி, மயக்கம், தலைச்சுழலல், மலபந்தம், பிரமேகம் இவை போம்.

வழக்குமுறை:

- **நெல்லிக்காயைத் துவையல் செய்து சாப்பிட சுவையின்மை, வாந்தி இவைகளை போக்கும்.**
- **நெல்லிவற்றலைக் குடிநீர் செய்து சாப்பிட மயக்கம், தாகம், ஓக்காளம் இவை நீங்கும்.**
- **மூப்படைந்த வரும் இளமையுடைய மாப்பிளைப்போல் அழகுடன் இருக்க வேண்டின், நெல்லிக்காயை பாகஞ் செய்து உண்ணுதல் வேண்டும்.**
- **இலைகொழுந்தை அரைத்து மோரில் கலந்து சீதக்கழிச்சலுக்குக் கொடுக்கலாம்.**
- **நெல்லிவற்றலை குடிநீர் செய்து சீனி கூட்டிப் பால் சேர்த்துச் சாப்பிட சூடு, ஆண்குறிப்புண்,வாந்தி தீரும்.[28]**
- **நெல்லிவற்றலை குடிநீர் செய்து தினமும் இரு வேளை கொடுத்து வர இரத்தமின்மை, காமாலை, பித்தகுன்மம் போம்.**

நெல்லிக்காய் சேர்ந்த பிறமருந்துகள்:

1.பிருங்காமலக தைலம்[9]

பிரயோகம்	**:**	**ஸ்நானம் செய்ய**
தீரும்நோய்கள்	**:**	**கண்ணெரிச்சல்,தேகஎரிவு,கண்ணோவு.**

2. ஊழி மாத்திரை[29]

அளவு	**:**	**குன்றியளவு (130 mg)**
தீரும்நோய்கள்	**:**	**ஊழி,அதிசாரம்,கிராணி.**

3.சீரகச்சூரணம்

அளவு	**:**	**வெருகடியளவு**
அனுபானம்	**:**	**சர்க்கரை**
தீரும்நோய்கள்	**:**	**வாந்தி,அக்கினிமந்தம்,உஷ்ணம்.**

4.சுண்டைவற்றல் சூரணம்

அளவு	**:**	**வெருகடியளவு**
அனுபானம்	**:**	**எருமைதயிர்**
தீரும்நோய்கள்	**:**	**பொருமல்,மந்தம்,மூலம்,கிரகணி**

5.கந்தக இரசாயணம்

அளவு	:	10-15 குன்றியளவு (1.3-2g)
அனுபானம்	:	சர்க்கரை,தேன்,நெய்
தீரும்நோய்கள்	:	மேகவியாதிகள்,மூத்திரகிரிச்சரம்,குட்டம், வாதம்.

6.திப்பிலி இரசாயணம்

அளவு	:	வெருகடியளவு
அனுபானம்	:	தாம்பிரச்செந்தூரம்
தீரும்நோய்கள்	:	சேத்துமம் 96, இளைப்பு, சயம்,காசம்.

7.பிரமி நெய்

அளவு	:	1\4 - 1\2 பலம்(8-16mg)
தீரும்நோய்கள்	:	பித்தாதிக்கம், சூதகசன்னி

8.நீர்முள்ளிகுடிநீர்

அளவு	:	1\2 ஆழாக்கு(84ml),2 வேளை
தீரும்நோய்கள்	:	சோபை, நீர்க்கட்டு

9.மண்டூராதி குடிநீர்

அளவு	:	1\4 ஆழாக்கு(42ml),2 வேளை
தீரும்நோய்கள்	:	பாண்டு,சோபை,காமாலை,மகோதரம்.

10.நெல்லிக்காய் இளகம்[30]

அளவு	:	புன்னைக்காயளவு
தீரும்நோய்கள்	:	பாண்டு,காமாலை,அழற்சி,வறட்சி,திமிர்வாயு, குன்மம்,உடம்பெரிவு.

NELLIKAI *(Phyllanthusemblica)*

DESCRIPTION:

Emblica is a small to medium-sized deciduous tree native to tropical southeastern Asia. Its leaves are simple, feather-like, and closely set along the branchlets. Flowers are green-yellow, and the round, greenish-yellow fruits are smooth and hard in appearance. The fruits, which ripen in autumn and are harvested by hand, are commonly used in the Indian diet.

SYNONYMS

Eng	:	Indian gooseberry
Mal	:	Nellikay
Sans	:	Amalaki
Hind	:	Amlika

TAXONOMICAL CLASSIFICATION:

Kingdom	:	Plantae
Clade	:	Angiosperms
Order	:	Malpighiales
Family	:	Phyllanthaceae
Genus	:	Phyllanthus
Species	:	Emblica

PART USED: Whole plant

CHEMICAL CONSTITUENT:

The fruit is rich in Vit-C (70-72%) and phyletic acid, gallic acid, lipid, flavonoids, colloidal complexes and micric acid.

The fruit pulp contain: moisture- 81.2, protein – 0.5, fat – 0.1, mineral matter – 0.7, fiber – 3.4, carbohydrate – 14.1, Ca- 0.05, P – 0.02%, Fe – 1.2/100g, nicotinic acid – 0.2mg/ 1.00g, vitamin C – 600 mg/ 100g.The fruit is rich source of pectin.[31]

ACTION:

The plant has Astringent, Refrigerant, Laxative, and Diuretic actions.

USES:

- Amla fruit is probably the richest known natural source of vitamin C. The fruit juice contains nearly 20 times as much vitamin C as orange juice.
- The fruit is used successfully in the treatment of human scurvy.
- Dried fruit is useful in haemorrhage, diarrhoea and dysentry.
- In combination with iron it is used as a remedy for anaemia, jaundice and dyspepsia.[11]
- Fermented liquor prepared from the fruit is used in jaundice, dyspepsia and cough.
- Acute biliary dysentry may be arrested by drinking a sherbet of amla with lime juice.
- The seeds are used in the treatment of asthma, bronchitis.
- The fruit is used in the preparation of writing inks and hair dyes.
- The fixed oil extracted from the fruits is reported to have the property of promoting hair growth.
- The fruit also used as an external application for inflammation of the eyes.[28]

Scientific validation:

Toxicity study:

This article reveals the safety of the amla.In toxicity studies in rats no toxicity was observed in single and longer-term–dose administration. Additionally no detrimental effect was noted on the liver or renal function[32]. No chromosomal aberrations were found following 7- and 14-day treatment regimens in rats with crude fruit extract[33].In another experiment no toxicity or mutagenicity was observed in rats even at the highest doses.[34]

Antioxidant activity:

This article showed the antioxidant property of the amla.Which has a rich source of vitamin C is considered to be effective in slowing down the ageing process. Ageing is a cumulative result of damage to various cells and tissues mainly by oxygen free radicals. Vitamin C is a scavenger of free radicals which breaks them down; it has an antioxidant synergism with vitamin E which prevents pre-oxidation of lipids.

Many papers are published about the magical effects of amla. However little is known about the chemistry and biological activity of its major constituents hydrolysable tannins (10-12 % in pericarp) except that they contained gallic acid and ellagic acids that inhibit the degradation of vitamin C and had some pharmacological activity entirely unrelated to the clinical use of fruits.[35]

A herbo-mineral formulation of the Ayurveda medicine named Peptic are composed of *Emblica officinalis, Glycyrrhiza glabra* and *Tinosporacordifolia* was tested for its anti-ulcer anti-oxidant activity in rats. Reports were made that Pepticare exhibit antiulcer activity which can be attributed to its anti-oxidant property.[36]

Immunomodulatorproperty:

This article exposed the immunomodulatory effect of amla.Immu-21 is an Ayurvedic polyherbal formulation containing extracts of *Emblica Officinalis, Ocimum sanctum, Withania somnifera* and *Tinospora cordifolia*. Its immune modulatory activity was studied on the proliferative response of splenic leukocytes to T cell mitogens, concanavalin (Con)-A and phytohemagglutinin (PHA) and B cell mitogen, lipopolysaccharide (LPS) in vitro by [3H]-thymidine uptake assay in mice. Pretreatment with Immu-21 selectively elevated the proliferation of splenic leukocyte to B cell mitogen LPS and cytotoxic activity against K 562 cells in mice.[37]

Hypolipidemic effect:

This article offered the hypolipidemic effect of amla.Flavonoids derived from *E.officinalis* exhibit maximum beneficial action by eliciting highly potent hypolipidaemic and hypoglycemic activities. In addition to this flavonoids were found to be effective in elevating the haemoglobin levels in rats[38]. It is also reported to be as antitumor[39].

Triphala containing one of the ingredients as *E.officinalis* is used to treat diseases such as anaemia, fever, chronic ulcers, constipation, jaundice and asthma. Polyphenolic fractions isolated from Triphala exhibit antimutagenic effect[40]. Active principles of Triphala was further evaluated and used as an excellent therapeutic formulation for infected wounds[41].

In Skin Sores and Wounds:

This paper reveals the productive effect of amla in skin.The milky juice of the leaves is a good application to sores. Grind the bark of *E.officinalis* (10g) into a paste and apply to the

cut or wound area once daily for 2 to 3days. Alternatively squeeze the leaves and extracts the juice to the cut once daily for 3 to 4 days. Healing occurs when the dynamic harmony of the doshas is restored[42].

In Dental disease:

This article deals with the detal care of the amla root.The roots (10 g) are ground and taken twice daily for one day only after taking food. Alternatively the leaves of are squeezed and the juice extracted. This juice is put in the ear (a few drops) to find relief from toothache.[43]

In Hair Growth:

This paper showed amla uses in hairgrowth.It boosts absorption of calcium thus creating healthier bones, teeth, nails and hair. It also helps maintain youthful hair colour and retards premature greying and supports the strength of the hair follicles so there is less thinning with age 55. The crushed fruits have a good effect on hair growth and prevent hair greying.[44]

In Ophthalmic Disorder:

This article deals with the purpose of amla in eye related diseases.The clinical trial was conducted in patients suffering from different ophthalmic disorders namely conjunctival xerosis, conjunctivitis, acute dacryocystitis, degenerative conditions and postoperative cataract patients with a herbal eye drop preparation. In most cases the improvement was observed with the treatment of the herbal eye drop.

During the course of study no side effects were observed and the eye drop was well tolerated by the patients. Ophtha care exhibit beneficial role in a number of inflammatory, infective and degenerative ophthalmic disorders.[45]

In Skin cancer:

The cancer preventive effect of *E.officinalis* was investigated on a two-stage process of skin cancer induced by7, 12dimethylbenz (a) anthracene (DMBA)in Swiss albino mice. It showed significant chemo-preventive effects on DMBA initiated and croton oil (1% in 100µl of acetone)promoted skin cancer development. *P. emblica* exhibited a significant reduction in tumour incidence, tumour yield, tumour burden and a cumulative number of papillomas. These findings were indicative of the chemopreventive potential of*E.officinalis*against skin carcinogenesis[46].

In Diabetes:

This paer showed the amla uses in diabetic patiants.Oral administration of the extracts (100 mg/kg body weight) reduced the blood sugar level in normal and in alloxan (120 mg/kg) diabetic rats significantly within 4 hours. *Emblica officinalis* and an enriched fraction of its tannoids are effective in delaying development of diabetic cataract in rats[47]. Aldose reductase (AR) has its involvement in the development of secondary complications of diabetes including cataract. *Emblica Officinalis* is proved as an important inhibitor of AR. Exploring the therapeutic value of natural ingredients that people can incorporate into everyday life may be an effective approach in the management of diabetic complications[48].

It decreases the fasting and 2-hour postprandial serum glucose level. It was demonstrated in a clinical study using both healthy and type 2 diabetic volunteers. One to 3 g of powdered dried fruit was consumed daily in 30 mL of water for 21days.[49]

3.3.கீழ்க்காய்நெல்லி

வேறுபெயர்:

கீழ்வாய்நெல்லி

கீழாநெல்லி

மாலறுது

மாலினி

பெருபுத்திரா

தூபாலாபூதாத்திரி

பெருவிரியகா[50]

பயன்படும் உறுப்பு : சமூலம்

சுவை : துவர்ப்பு,கைப்பு,புளிப்பு,இனிப்பு

தன்மை : தட்பம்

பிரிவு : இனிப்பு

செய்கை:

வீக்கமுருக்கி - Deobstruent

சிறுநீர்ப்பெருக்கி - Diuretic

துவர்ப்பி - Astringent

குளிர்ச்சியுண்டாக்கி - Coolent[28]

குணம்:

"சீதமதி பித்தவிலடஞ் செவ்விழியின் னோய்க் கூட்டம்

பூதமொடு பேயிரத்தப் போக்குகளும் - பூதலத்துள்

தாழ்வாய்ப் பணிந்தேகுந் தப்பாது பொய்யலவே

கீழ்வா யெனுநெல்லிக் கே". (அ.கு)

இதனால் அசுத்த ரத்தம், விழிநோய், நாவறட்சி, தாபம் இவைகளையும் நீக்கும்.வாதத்தைப் பெருக்கும்.

வழக்குமுறை:

- பல பிணிகளினால் நொந்து உடல் மெலிந்து வெளுத்திருக்குங்கால்,கீழாநெல்லியை அரைத்து , நல்ல பசுவின் தயிரில் கலந்து, நாளும் கற்பமுறையின்படி உண்ண ,மேகவெட்டை முதலிய நோய்கள் யாவும் போம்.

- இப்பூண்டின் இலை கொழுந்தைக் குடிநீரிட்டுச் சீதகழிச்சலுக்குக் கொடுக்கலாம்.

- கற்கம் : இதன் சமூலத்தை அரைத்து ஒரு கொட்டைப் பாக்குப் பிரமாணம் பசுவின் பாலில் கலக்கி சாப்பிட்டு வர சோகை , காமாலை, பாண்டு, வாதபித்த ரோகங்கள் குணமாகும்.

- இரத்தம் அதிகப்படும், கண் குளிரும்.

- வேரை கழுநீரில் அரைத்துக் கலக்கி சாப்பிட பெரும்பாடு போம்.

- வேரைப் பச்சையாய் 17 கிராம் எடுத்து அரைத்து பாலில் கலக்கிக் கொடுக்க காமாலை போம்.

- இலை , வேர் இரண்டையும் குடிநீரிட்டுச் சுரங்களுக்குச் சூட்டோடே கொடுக்க காய்ச்சல் தணியும்.[28]

கீழாநெல்லி சேர்ந்த பிறமருந்துகள்:

1.பஞ்சகவ்விய லேகியம்[9]

அளவு	:	நெல்லிக்காயளவு
தீரும்நோய்கள்	:	தீராதபெரும்பாடு,சூதகவலி,குன்மம்.

2.திப்பிலிகிருதம்

அளவு	:	1-2 தேக்கரண்டி
தீரும்நோய்கள்	:	சுரம்,விக்கல்,அரோகம்,தலைநோய்.

3.இராஜசிந்தாமணி எண்ணெய்

அளவு	:	1\4 - 1\2 பலம்(8-16ml)
தீரும்நோய்கள்	:	கைகால்பிடிப்பு,கிரந்தி,கொறுக்கு.

4.வருணலோக செந்தூரம்[10]

அளவு	:	1\4- 11\2 குன்றி(32.5-195mg)
அனுபானம்	:	தேன்,நெய்
தீரும்நோய்கள்	:	பாண்டு,காமாலை.

5. கரிசாலை இளகம்[29]

அளவு : புன்னையளவு
தீரும்நோய்கள் : பாண்டு,பித்தவெட்டை,எரிவு,கிராணி

6.ஆடாதொடை நெய்

அளவு : காசெடை(165mg)
தீரும்நோய்கள் : நாட்பட்டசுரம்.

7.நொச்சி தைலம்

அளவு : காசளவு(165mg)
தீரும்நோய்கள் : 5 வகை காசம்,கயம்.

8. பஞ்சகௌவியக்கிருதம்[10]

அளவு : 1-2 தேக்கரண்டி
தீரும்நோய்கள் : பெரும்பாடு,குன்மம்,பீநிசம்,பித்தப்பாண்டு, காமாலை.

9.கீழாநெல்லி தைலம் [28]

பிரயோகம் : ஸ்நானம் செய்ய
தீரும்நோய்கள் : உட்சுரம்,அழலை,கை ,கால், கண் எரிச்சல்,நடுக்கல்,தலைசுழற்றல், வாந்தி.

10.கீழாநெல்லி கற்கம்

அளவு : கொட்டைப்பாக்களவு
அனுபானம் : பசும்பால்
தீரும்நோய்கள் : காமாலை,சோபை,பாண்டு, வாதபித்தரோகங்கள்.

KEEZHANELLI (*Phyllanthus niruri*)

DESCRIPTION:

It grows 50 – 70 cm tall and bears ascending herbaceous branches. The bark is smooth and light green. It bears numerous pale green flowers which are often flushed with red. The fruits are tiny, smooth capsules containing seeds.

SYNONYMS

Eng : Indian Phyllanthus

Mal : Keezhanelli

Sans : Bhumyamalaki

Hind : Bhutan-anvalah

TAXONOMICAL CLASSIFICATION:

Kingdom : Plantae

Clade : Angiosperms

Order : Malpighiales

Family : Phyllanthaceae

Genus : Phyllanthus

Species : Niruri / amarus

PARTS USED: Whole plant

CHEMICAL CONSTITUENTS:

Phyllanthin, hypophyllanthin, phyllanthus D, geranin, rutin, corilagin, linnanthin, amarulone, epibubbialine and isobubbialine.

In leaf contain : moister content 9.00 – 9.10%, protein 18.77 – 19.00%, carbohydrate – 56.97 – 57.02%, fiber 9.13 – 9.29%, Fe 7.57 – 7.89, Zn 0.27 – 0.30, Mg 40.00 – 45.0,Ca 178.33 – 182.10, ascorbic acid 18.80 – 19.12mg/100g.K 45.00 – 50.10mg/100g.[51]

ACTION:

The plant has Deobstruent, Diuretic, Astringent, Coolent actions.

USES:

- It is used in stomach troubles such as dyspepsia, colic, diarrhoea and dysentry and also employed in dropsy and diseases of the urinogenital system.
- Fresh roots are said to be beneficial for jaundice. They are taken with milk as a galactagogue.
- A decoction of the leaves is used as a refrigerant for the scalp.
- Leaves and roots are made into the poultice with rice water for application on oedematous swellings and ulcers.
- The latex is also applied to offensive sores and ulcers and mixed with oil it is used in ophthalmia.
- It helps to remedy for fatty liver and liver damage due to any reason. It promotes liver action.
- It is also said to be used in the case of anorexia.[11]
- The infusion of the root and leaves is a good tonic and diuretic when taken cold in repeated doses.
- A poultice of the leaves with salt cures scabby disorder of the skin.
- Infusion of young shoots is an effective cure to treat dysentery.
- Fresh juice of the whole plant along with clarified butter is very effective in the treatment of menorrhagia, leucorrhea and gonorrhoea.
- The plant said to be used in diabetes.

Scientific validation:

Toxicological assessment:

This article reveals the safety of *P. amarus.* There was no mortality among the animals and they did not show any toxicity or behavioural changes at the dose level of 2000 mg/kg. These findings suggest that *Phyllanthus amarus* was safe and non-toxic to rats up to 2000 mg/kg in acute toxicity study.[52]

Antiviral activity:

This paper deals the antiviral property of Keezhanelli.The aqueous extract of *P. amarus* was tested for its activity against WSSV in marine shrimp and freshwater crabs. *P.amarus* showed antiviral activity against WSSV at the concentration of 150 mg/kg of animal body weight.[53]

An aqueous extract on human hepatocellular carcinoma derived cell at1 mg/ml concentration on a single dose. Inhibition of secretion of HBsAg for a period of 48 h was observed.[54]

Immunomodulator effect:

This article shows the immunomadulatory effect of *P. niruri*.Thirty-two male Wistar rats of average body weight of 85.5 ± 4.55 g were grouped into four (AD). Group A received distilled water (control) while doses of 250, 500 and 1000 mg/kg body weight of extract were orally administered once daily for 84 days to animals in groups B, C and D respectively. The result shows reduce the body weight and blood glucose level at the same time it increases the serum interleukin-6 and tumour necrosis factor-αconcentrations reduced and the lipid profile level was reduced and the WBC count was increased. The result of the study established scientifically the folklore use of the aqueous leaf extract of *P. amarus* as blood tonic for the prevention and or cure of infective and degenerative diseases.[55]

Antiamnesic effect:

This article showed the antiamnesic effect of Keezhanelli.*Phyllanthus amarus* (PAs) 50, 100 and 200 mg/kg produced a dose-dependent improvement in memory scores of young and older mice. PAs also reversed successfully the amnesia induced by scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.). The brain cholinesterase activity was also

reduced. The underlying mechanism of action for the observed nootropic effect may be attributed to pro-cholinergic activity exhibited by PAs.[56]

Hepatoprotective activity:

This paer reveals the hepatoproductive activity of *P. amarus*.In-vivo methanolic and aqueous extracts of the seeds of *P. amarus* 250mg/kg were found to have protective properties in rats with CCl_4 induced liver damage as judged from serum biochemical enzyme marker activities and histopathological studies.*P. amarus* (1–4 mg/ml) shows the beneficial roles against ethanol-induced liver injury in rats. The possible mechanism may involve their antioxidant activity.[57]

Antimicrobial activity:

This paper explain the antimicrobial effect of *Phyllanthus.*The strains isolated from both HIV seropositive patients were susceptible to various concentrations of the *P. amarus* extracts (5, 10, 20, 40 and 80 mg ml-1) which were assessed against extend spectrum β-lactamase producing *Escherichia coli* isolated from the stool samples of HIV seropositive patients with or without diarrhoea.[58]

Hypolipidemic effect:

This paper deals the hypolipidemic property of Keezhanelli.Hydro-alcoholic extract of leaves of *Phyllanthus amarus* Schumacher (HAEPAS) was studied for its invivo anti-hyperlipidemic potential using cholesterol diet-induced hyperlipidemia model in rats. The result of the study indicated that HAEPAS possess significant hypolipidemic activity at doses 300 and 500 mg/kg.[59]

Antioxidant property:

This article showed the antioxidant acitivityof the *Phyllanthus.*The aqueous extract (PAAEt) treated rats showed a significant decrease in plasma LPO and a significant increase in plasma vitamin C, uric acid, GSH levels and GPx, CAT and SOD activities. Single cell gel electrophoresis experiment reveals that PAAEt was devoid of genotoxicity and had a significant protective effect against H_2O_2, STZ and nitric oxide (NO) induced lymphocyte DNA damage.[60]

Hypotensive effect:

This paper establishes the hypotensive effect of Keezhanelli.The effect of the aqueous extract of the leaves of *P. amarus* on blood pressure was evaluated in normotensive male rabbits. Intravenously administered aqueous doses (5 mg to 80 mg/kg) of the extract to anaesthetized normotensive male rabbits produced a significant fall in mean diastolic, systolic and mean arterial pressures in a graded dose-response manner. The dose of 5 mg/kg produced the least hypotensive effect causing a fall in mean diastolic, systolic and mean arterial pressure of 13.3 ± 3.1, 19.7 ± 5.4 and14.3 ± 3.4 mmHg respectively while the dose of 80 mg/kg produced the greatest fall in mean diastolic, systolic and mean arterial pressure of 49.7 ± 7.9, 45.5 ± 9.5 and 48.00 ± 6.5 mmHg respectively. [61]

Antispasmodic effect:

This paper show the muscular function of the Keezhanelli.The ether extract obtained from *P. niruri* was found to be the most active as an antispasmodic being about 6- to 27-fold more effective in causing antispasmodic actions in vascular and nonvascular preparations than other extracts.[62]

3.4. கரிசலாங்கண்ணி

வேறுபெயர்:

கரிசனாங்கண்ணி,
கரிசாலை,
கரியசாலை,
கைகேசி,
கைவீசி இலை,
கையாந்தகரை,
பிருங்கராஜம்,
கரிப்பான்,
கையான்,
தேகராஜம்.

வகை	:	**நீலம், மஞ்சள், சிகப்பு, வெள்ளை**
பயன்படும் உறுப்பு	:	**பூண்டு**
சுவை	:	**கைப்பு**
தன்மை	:	**வெப்பம்**
பிரிவு	:	**கார்ப்பு**

செய்கை:

பித்தநீர்ப்பெருக்கி	-	Cholagogue
உரமாக்கி	-	Tonic
உடற்தேற்றி	-	Alterative
வாந்தியுண்டாக்கி	-	Emetic
நீர்மலம்போக்கி	-	Purgative
வீக்கமுருக்கி	-	Deobstruent
ஈரல்தேற்றி	-	Hepictonic

குணம்:

"குரற்கம்மற் காமாலை குட்டமொடு சோபை
யுறற்பாண்டு பன்னோ யொழிய- நிரற்சொன்ன
மெய்யாந் தகரையொத்த மீளி ண்ணு நற்புலத்துக்
கையாந் தகரையொத்தக் கால்". (அ.கு)

இதனால் குரலுறுப்பு நோய், காமாலை, குட்டம், வீக்கம், பாண்டு, பல்நோய் ஆகியவை போம்.உடலுக்கு பொற்சாயலும், பலமும் உண்டாகும்[28].

வழக்குமுறை:

- கரிசலைச் சூரணத்தை அயச்செந்தூரத்திற்கு அனுபானமாகக் கொள்ள பாண்டு,சோபை,காமாலை முதலிய நோய்கள் தீரும்.

- வேர்ப்பொடித்ததை, கல்லீரல் மண்ணீரல் நோய்களுக்கும், தோலைப் பற்றிய பிணிகளுக்கும் கொடுக்கலாம்.

- மஞ்சள் கரிசலை கறியாகச் செய்துண்ண அறிவின் தெளிவும் திருவும் சேரும்.

- இலைச்சாற்றை நல்லெண்ணெய் அல்லது தேங்காய் எண்ணெயில் காய்ச்சித் தலைக்குத் தேய்த்து வர முடிகறுத்துத் தழைத்து வளரும்[28].

- கரிசாலை இலையை சிறிது மிளகுடன் கூட்டி அரைத்து தினமும் இரு வேளை சுண்டைக்காய் பிரமாணம் சாப்பிடபாண்டு, சோபை, காமாலை முதலிய நோய்கள் தீரும்.

- கரிசாலைச் சாற்றை ஒன்று அல்லது இரண்டு தேக்கரண்டி வீதம் மோரில் கலக்கி சாப்பிடலாம்[63].

கரிசாலை சேர்ந்த பிறமருந்துகள்

1.ஜோதிரச மாத்திரை[9]

அளவு	:	**7-8 மாத்திரை**
அனுபானம்	:	**சுக்குகியாழம்**
தீரும்நோய்கள்	:	**மலசலக்கட்டை உடைக்கும்**

2.அஷ்டபைரவமாத்திரை

அளவு	:	**1 மாத்திரை (2 வேளை, 2 நாள்)**
அனுபானம்	:	**முலைப்பால்,தேன்**
தீரும்நோய்கள்	:	**சுரம், சந்நி, தோஷம்,இசிவு**

3. எமதண்டக்களிகை

அளவு	:	**1 மாத்திரை**
அனுபானம்	:	**தேன்**
தீரும்நோய்கள்	:	**சுரம், சன்னி, தோஷம்**

4. திப்பிலிலேகியம்

அளவு	:	**சுண்டைக்காய் பிரமாணம்**
தீரும்நோய்கள்	:	**ஈளை,இருமல்,காசம்,அரோகம்,கூஷயம்**

5.இஞ்சிலேகியம்

அளவு	:	**கழற்சிபிரமாணம்**
தீரும்நோய்கள்	:	**ஏப்பம்,நெஞ்செரிவு,வாய்குமட்டல்.**

6.கூழ்பாண்டகிருதம்

அளவு	:	**2-3 தேக்கரண்டி**
தீரும்நோய்கள்	:	**பிரமேகம்,கிரிச்சுரம்,தேகஎரிவு,வறட்சி.**

7.பொன்னாங்கண்ணி தைலம்

அளவு	:	**2-3 தேக்கரண்டி**
தீரும்நோய்கள்	:	**வெட்டை, மூலம், பிரமேகம்,மேகக்காங்கை.**

8.மாந்த எண்ணெய்

அளவு	:	2-3 தேக்கரண்டி
அனுபானம்	:	முலைப்பால்
தீரும்நோய்கள்	:	சகலமாந்தமும் தீரும்.

9. கருடன்கிழங்கு எண்ணெய்

அளவு	:	1\4 - 1\2 அவுன்ஸ்
தீரும்நோய்கள்	:	மேகம்21,படை,வாயு,மேகரணம்,சொறி, சிரங்கு.

10.அயச்செந்தூரம்

அளவு	:	குன்றியெடை(130mg)
அனுபானம்	:	நெய்,தேன்
தீரும்நோய்கள்	:	பாண்டு, சோபை, காமாலை[10]

KARISALAI *(Ecliptaalba)*

DESCRIPTION:

Eclipta alba (Asteraceae) is an annual herbaceous plant commonly known as a false daisy. It is an erect or prostrates much branched roughly hairy annual rooting at the nodes; the leaves are opposite, sessile and lanceolate. It is also known as Bhringaraj and Karisilakanni which is found a common weed throughout India ascending up to 6000 ft. The genus name comes from the Greek word meaning "Deficient" with reference to the absence of the bristles and awns on the fruits. The specific *Eclipta alba* means white which refers to the colour of the flowers.

SYNONYM:

Eng : Trailing eclipta

Mal : Tanjung

Sans : Bhringaraj

Hind : Bungah

TAXONOMICAL CLASSIFICATION:

Kingdom	:	Plantae
Clades	:	Angiosperms
Order	:	Asterales
Family	:	Asteraceae
Genus	:	Eclipta
Species	:	Alba

PART USED: Whole plant [28]

CHEMICAL CONSTITUENT:

It contains alkaloids, flavonoids, stigmasterol, triterpenoids, glucosides, wedelolactone, demethylwedelolactone and dimethyl wedelolactone 7 glycosides.

ACTION:

The plant has Cholagogue, Tonic, Alternative, Purgative, Deobstruent, Hepatotonic action.[64`]

MINERAL ELEMENTS:

The minerals Na, Mg, Al, K, Ca, Fe, Cr, Mn, Co, Ni, Cu, Zn and Ag were detected in the plant. The voltammetric analysis carried out on leaves showed that the concentrations of macronutrients such as Ca, P, Mg, K, Fe and S ranged from 9.62-41.74, 1.00-8.630, 3.53-35.50, 12.04-56.28, 0.111-3.845 g/kg and 1.124-5.843 mg/kg.[11]

USES:

- The plant is used in indigestion, diarrhoea, dysentry, and digestive diseases.
- Roots and leaves are largely used alone or in combination with ajowan seeds in derangement of the liver and gallbladder.
- It is used as a liver tonic.
- It is highly effective for a urinary tract infection.
- Leaves are very effective for skin disorders, skin allergies and cracked heels.
- It helps in rejuvenate the body.
- Leave extract is also used for a toothache.
- It increases the learning and memory ability.
- It is used in hair dyes and for tattooing purpose.
- It is very effective for eyesight and helps to the purification of blood.
- It promotes hair growth and prevents hair fall, premature greying.
- Leaves are used for eczema, ringworm and leucoderma.[11]

Scientific validation:

Toxicity studies:

In studies conducted the alcoholic extract of *E.alba* shows no signs of toxicity in rats and mice and the minimum lethal dose was found to be greater than 2.0g/kg when given orally and intraperitoneally in mice[65].

Hepatoproductive activity:

This review deals the hepatoproductive effect of *alba*.The hepatoprotective effect of the ethanol/water (1:1) extract of *Eclipta alba* has been studied at subcellular levels in rats against CCl_4-induced hepatotoxicity. *E.alba* significantly counteracted CCl_4-induced inhibition of the hepatic microsomal drug metabolizing enzymes. The loss of hepatic lysosomal acid phosphatase and alkaline phosphatase by CCl_4 was significantly restored by *E.alba*. The study shows that hepatoprotective activity of *Eclipta alba* is by regulating the levels of hepatic microsomal drug metabolizing enzymes.[66]

Antihyperlipidemic properties:

This paper deals the antihyperlipidemic action on *E. alba*.It has been reported that in the atherogenic diet-induced hyperlipidemic model the aqueous leaf extract of the *Eclipta prostrata* was given orally to the rats which significantly reduced total cholesterol, triglycerides, total protein. There was a significant elevation in the high-density lipoprotein cholesterol levels. 200mg/kg of extract showed better results compared to 100mg/kg.[67]

Immunomodulatory activities:

This review point out the immunomodulatory activity of*E.alba*.Experimentation made to assess the immunomodulatory activity of methanol extracts of whole plant of *E. alba* (1.6% wedelolactone) at five dose levels (dose-response relationship) ranging from 100 to 500 mg/kg using carbon clearance, antibody titer and cyclophosphamide immunosuppression parameters significantly increased phagocytic index and antibody titer and the F ratios of the phagocytic index and WBC count were also significant.[68]

Anti-inflammatory activity:

These papers describe the anti inflammatory effect of *E. alba*. Wistar albino rats were used to investigate anti-inflammatory activity in which methanolic extract was administered orally. 100 and 200 mg/kg showed significant anti-inflammatory activity in carrageenin and egg white induced hind paw oedema in rats which were compared with indomethacin (10 mg/kg) and cyproheptadine (8 mg/kg).[69]

Analgesic activity:

This article show the analgesic effect of *E. alba*.Analgesic effect was studied on albino mice using an ethanolic and alkaloidal extract of *Eclipta alba*. Standard experimental models such as the tail clip method, the tail flick method and the acetic acid-induced writhing response were used which showed both the ethanol extract as well as the total alkaloids produced good analgesic activity in all the different models of analgesia used. The total alkaloidal fraction was the most efficacious in all models tested.[70]

Antidiabetic activity:

This review reveal the antidiabetic activity of *E. alba*.Leaf suspension (2 & 4g/kg) orally in alloxan-induced diabetic rats resulted in a reduction in blood glucose level, glycosylated haemoglobin. There was the decreased activity of glucose-6 phosphatase and fructose1, 6-bisphosphatase, and an increase in the activity of liver hexokinase. Thus oral administration of *Eclipta alba* suspension possesses potent antihyperglycemic activity.[71]

Hair growth:

This paper show the uses of *E. alba* in hairgrowth.It is used in hair oil preparations since it promotes hair growth and maintains hair black. 10%w/v of *Eclipta alba* was the main ingredient in the preparation of a herbal formulation for hair growth.[72]

Anticancer activity:

This review deals the anticancer property of the *E. alba*.Dasyscyphin-C (saponins) a newer isolated compound from *E.prostrata* reported having anticancer-cytotoxic activity. It was tested under in-vitro conditions in HeLa (Human cervical carcinoma) & Vero cell lines. At the concentration of 50µg/ml, it showed a good anticancer-cytotoxic activity on HeLa cells.[73]

3.5.தேன்

செய்கை:

உள்ளழலாற்றி,
மலமிளக்கி,
துவர்ப்பி,
அழுகலகற்றி,
கோழையகற்றி,
போஷணகாரி,
பசித்தீதூண்டி,
தூக்கமுண்டாக்கி.

தேனில் உடலுக்கு தேவையான இனிப்புச் சத்து, உலோகசத்துகள், வைட்டமின் போன்ற எல்லாச்சத்துகளும் சிறு அளவில் பொருந்தியிருப்பதாகத் தற்கால விஞ்ஞானிகள் கண்டுபிடித்திருக்கின்றாகள்.

நம் நூற்களில் தேன் 12 நாழிகையில் ஜிரணமாகிவிடுகின்றதென்று கூறப்பட்டுள்ளது.

வழக்குமுறை:

- இலேகியம், பாணிதம், மெழுகு, கட்டு, கண் மை போன்ற மருந்துகள் செய்யத் தேன் பயன்படுகிறது.
- பற்பம்,செந்தூரம்,சூரணம், மாத்திரை, குடிநீர் போன்றவைகளுக்கு சிறந்த துணைமருந்தாகும்.
- குழந்தைகளின் இருமலுக்கு தேன், எலுமிச்சைச்சாறுடன் கலந்து குறைந்த அளவில் கொடுத்துவரத் தணியும்.
- இளைத்த உடம்பினருக்கு இதயத்தைப் பலப்படுத்த தேனை வழங்கி வரலாம்.
- மதுமேக நோயிற்காக வழங்கப்படும் மருந்துகளில் தேன் முக்கியப் பொருளாகச் சேர்க்கப்படுக்கின்றது.
- கோழையை நீக்கும் , புண்ணாற்றும் மருந்துகளில் சேர்க்கப்படுகிறது.[2]

HONEY

Phytochemicals:

Honey is known to be rich in both enzymatic and non-enzymatic antioxidants, including catalase, ascorbic acid, flavonoids and alkaloids.[74,75,76]

Scientific validation:

Wound healing property:

This article deals the wound healing property of the honey.The pharmacological activity of honey balm, its influence on wounds healing processes and the rate of scar formation in comparison with control groups were assessed. Clinical and histopathological studies showed that honey balm not only shortened the period of wound healing but also had a positive impact on the general health condition of the animals.[77]

Antiproliferative Effect:

This review has clearly demonstrated certain honey polyphenols tested in laboratory setups showed to be a promising pharmacological agent for inhibiting cancer cell proliferation.[78]

Antifungal activity:

This paper revealed the antifungal activity of honey.The honey samples were examined for antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Microsporum gypseum*, *Candida albicans*, *and Saccharomyces* sp. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the honey were also determined. Results obtained reveal that the honey samples showed varying levels of inhibitory activity at various concentrations against the fungi tested with zones of inhibition increased with increasing honey concentration.[79]

3.6 வெல்லம்

வெல்லம் பித்தகுன் மத்தைப் போக்கும்.ஆனால் ஐயத்தையும், வயிற்றுப்புழுக்களையும், நீரிழிவு நோயையும் உண்டாக்கும்[28].

"குன்ம பித்தம் போக்குமதி கோழைதனை யுண்டாக்குந்
துன்மலத்துட் கீடத்தைத் தோற்றுவிக்கும்- நன்மைபோல்
மெல்லமது நீரை விளைவிக்கும் மாமதுர
வெல்லமென நாளும் விளம்பு".

(அ.கு)

Scientific validation:

This article reveals genotoxic effects induced by arsenic through parenteral administration and ameliorate by jaggery. Chromosomal aberrations were more pronounced in arsenic-treated mice, while supplementation of jaggery with arsenic reduced the incidence of the aberrations. The outcome of the study showed that Jaggery the natural functional food has the efficiency to encounter the genotoxic effects induced by arsenic.[80]

This article showed Fresh goat tissues (tongue) were fixed separately with buffered 10% formalin (positive control), honey, sugar syrup, jaggery syrup, and distilled water (negative control). 24 h fixation was done at room temperature followed by conventional processing and routine H and E staining. The stained sections were assessed for cytoplasmic and nuclear detail by three pathologists under the light microscope and were graded accordingly. The preservation of tissue by honey, sugar, and jaggery syrup was comparable to that of formalin. Among the three natural fixatives, jaggery syrup excelled. Hence, it can be considered as an equally effective formalin substitute.[81]

CHAPTER IV

MATERIALS AND METHODS

4.1. PREPARATION OF THE TEST DRUG:

4.1.1. COLLECTION OF RAW DRUGS:

Ayam was collected from Trichy river bed and other herbal drugs such as Keezhaneli,Nellikai and Karisalai were collected from the Tambaram market, Chennai.

4.1.2. AUTHENTICATION:

The mineral drug Ayam were identified and authenticated by Lecturer, Department of Gunapadam , National Institute of Siddha , Chennai-47.

The herbal drugs Nellikai (*Phyllanthus emblica*), Keezhkainelli(*Phyllanthus nirui*), Karisalai (*Eclipta alba*) were identified and authenticated by Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Chennai-47.

4.1.3.METHODS OF PURIFICATION:

1.Purification of Ayam:

The juice of jamun fruit (*Syzigium cumini*) was poured over the iron powder till the powder is immersed in the juice and then kept in sunlight until the juice completely dried after that the dried iron powder was washed. This process is repeated six times to get the purified iron, every time used fresh juice of *Syzigium cumini*[2].

2. Purification of Nellikai:

Remove the seeds from Nellikai.[82]

4.1.4. METHOD OF AYA PODI ELAGAM PREPARATION:

Ingredients:

Purified Ayapodi (Iron powder)	-	280 g
Nellikai chaaru (*Phyllanthus emblica* juice)	-	140 g
Keezhkainelli chamoolamchaaru (*Phyllanthus nirui* whole plant extract)	-	140 g
Karisalai chaaru (*Ecliptaalba* plant extract)	-	105 g
Purified honey	-	87.5 g
Jaggery (vellam)	-	70 g

Method of preparation:

Mix all the extracts mentioned above the purified iron was ground in a mortar for tweleve hours adding the mixture of the extracts. The resultant product was kept in an iron pot;mix it well in the remaining part of the extracts then heat the pot slowly. Stir well the iron paste with honey and jaggery (vellam).When cool, pour the paste into a porcelain vessel and keep it for use.

Therapeutic uses:

It cures Pithapandu (Anaemia), Pithavettai, and Manjal noi (Jaundice).

Therapeutic dosage:

The dose is 2.5 g to 5 g twice daily after food.

Duration: 1 Mandalam (48 days) [10]

INGREDIENTS OF AYAPODI ELAGAM

Nellikai

Karisalai

Keezhanelli

Vellam

Honey

Before Purification of Ayam

Purification process of Ayam

After Purification of Ayam

Medicine Preparation

Ayapodi Elagam

4.2. ANALYTICAL STUDY OF AYAPODI ELAGAM

The Ayapodi Elagam was subjected to following analytical studies like physicochemical analysis, Biochemical Analysis, Phytochemical analysis and Spectroscopic analysis by using sophisticated instruments.[83]

4.2. STANDARDIZATION OF AYAPODI ELAGAM

4.2.1. QUALITATIVE ANALYSIS PHYSICO-CHEMICAL ANALYSIS OF AYAPODI ELLAGAM

The physico- chemical properties of Ayapodi Elagam was carried as per standard procedure at The Tamilnadu Dr.M.G.R.Medical University, Guindy, Chennai -32.

1. Loss on Drying:

An accurately weighed 2g of Ayapodi Elagam formulation was taken in a tarred glass bottle. The crude drug was heated at 105^0C for 6 hours in an oven till a constant weight. Percentage moisture content of the sample was calculated with reference to the shade dried material.

2. Determination of total ash:

Weighed accurately 2g of Ayapodi Elagam formulation was added in crucible at a temperature 600^0C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

3. Determination of acid insoluble ash:

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffler furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.

4. Determination of water soluble ash:

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450^0C in a muffle furnace. The amount of soluble ash was determined by drying the filtrate.

5. Determination of water soluble Extractive:

5gm of air dried drug, coarsely powered Ayapodi Elagam was macerated with 100ml of distilled water in a closed flask for twenty-four hours shaking frequently. Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100^0 C and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

6. Determination of alcohol soluble extractive:

2.5 gm. of air dried drugs; coarsely powdered Ayapodi Elagam was macerated with 50 ml alcohol in closed flask for 24 hrs. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^0C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

4.2.2. BIO-CHEMICAL ANALYSIS:

The bio-chemical analysis of Ayapodi Elagam (A.E) was done in Biochemistry lab, National Institute of Siddha, Chennai-47.

Preparation of Extract:

5gm of Ayapodi Elagam (A.E) was weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was allowed to cool and filtered in a 100ml volumetric flask and made up to 100ml with distilled water.

S.NO	EXPERIMENT	OBSERVATION
1.	Appearance of sample	Black in colour
2.	Test for Silicate: a. A little (500mg) of the sample is shaken well with distilled water. b. A little(500mg) of the sample is shaken well with con. HCl/Con. H2So4	Sparingly soluble
3.	Action of Heat: A small amount (500mg) of the sample is taken in a dry test tube and heated gently at first and then strong	White fumes evolved
4.	Flame Test: A small amount (500mg) of the sample is made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame	No bluish green flame appeared
5.	Ash Test: A filter paper is soaked into a mixture of sample and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited	No Yellow colour flame appeared

I. Test for Acid Radicals on Ayapodi Elagam

S.NO	EXPERIMENT	OBSERVATION
1.	Test For Sulphate: 2ml of the above prepared extract is taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	No Cloudy appearance present
2.	Test For Chloride: 2ml of the above prepared extracts is added with 2ml of dil-HCl is added until the effervescence ceases off...	No Cloudy appearance present
3.	Test For Phosphate: 2ml of the extract is treated with 2ml of dil. ammonium molybdate solution and 2ml of con.HNO	Cloudy yellow precipitate present
4.	Test For Carbonate: 2ml of the extract is treated with 2ml dil.magnesium sulphate solution	Presence of cloud appearance
5.	Test For Nitrate: 1gm of the substance is heated with copper turning and concentrated H2SO4 and viewed the test tube vertically down	No brown gas is evolved
6.	Test For Sulphide: 1gm of the substance is treated with 2ml of con. HCL	No rotten Egg Smelling gas is evolved
7.	Test For Fluoride & Oxalate: 2ml of extract is added with 2ml of dil. Acetic acid and 2ml dil. calcium chloride solution and heated.	Absence of Cloudy appearance
8.	Test For Nitrite: 3drops of the extract is placed on a filter paper, on that-2 drops of dil. acetic acid and 2 drops of dil. Benzidine solution is placed.	No characteristic changes
9.	Test For Borate: 2 Pinches (50mg) of the substance is made into paste by using dil. Sulphuric acid and alcohol (95%) and introduced into the blue flame.	No bluish green colour flame appeared

II.Test for basic radicals on Ayapodi Elagam:

S.NO	EXPERIMENT	OBSERVATION
1.	Test For Lead: 2ml of the extract is added with 2ml of dil. Potassium iodine solution	No yellow Precipitate is obtained.
2.	Test For Copper: a. One pinch (50mg) of substance is made into paste with con. HCl in a watch glass and introduced into the nonluminous part of the flame	No blue colour precipitate is formed.
3.	Test For Aluminum: To the 2ml of extract dil. sodium hydroxide is added in 5 drops to excess	Yellow colour Appearance
4.	Test For Iron: A. To the 2ml of extract add 2ml of dil. Ammonium solution B. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added	Brown precipitate is formed Red colour appearance
5.	Test For Zinc: To 2ml of the extract dil .sodium hydroxide solution is added in 5 drops to excess and dil. ammonium chloride is added.	White precipitate is formed
6.	Test For Calcium: 2ml of the extract is added with 2ml of 4% dil. ammonium oxalate solution	Cloudy appearance or white precipitate formation is present
7.	Test For Magnesium: To 2ml of extract dil. Sodium hydroxide solution is added in drops to excess	White precipitate is obtained
8.	Test For Ammonium: To 2ml of extract 1 ml of Nessler's reagent and excess of dil. Sodium hydroxide solution are added.	Brown colour is appeared
9.	Test For Potassium: A pinch (25mg) of substance is treated off with 2ml of dil. Sodium nitrite solution an then treated with 2ml of dil. Cobalt nitrate in 30% dil. Glacial acetic acid.	No yellowish precipitate is obtained.

10.	Test For Sodium: 2 pinches (50mg) of the substance is made into paste by using HCl and introduced into the blue flame of Bunsen burner	No yellow colour flame appeared
11.	Test For Mercury: 2ml of the extract is treated with 2ml of dil. sodium hydroxide solution.	No Yellow precipitate is obtained
12.	Test For Arsenic: 2ml of the extract is treated with 2ml of dil .sodium hydroxide solution	No brownish red precipitate is obtained

III. Miscellaneous test for Ayapodi Elagam:

S.NO	EXPERIMENT	OBSERVATION
1.	Test For Starch : 2ml of extract is treated with weak dil. iodine solution	No Blue colour Formation is present
2.	Test For Reducing Sugar: 5ml of Benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes are noted.	Brick red colour developed
3.	Test For The Alkaloids: a) 2ml of the extract is treated with 2ml of dil. Potassium iodide solution. b) 2ml of the extract is treated with 2ml of dil. Picric acid	Yellow precipitation appears
4.	Test For Tannic Acid: 2ml of extract is treated with 2ml of dil. Ferric chloride solution	No Black precipitate is obtained
5.	Test For Unsaturated Compound: To the 2ml of extract 2ml of dil. Potassium permanganate solution is added	Potassium permanganate is not decolourised
6.	Test For Amino Acid: 2 drops of the extract is placed on a filter paper and dried well. 20ml of Biurette reagent is added.	Violet colour is not developed
7.	Test For Type Of Compound: 2ml of the extract is treated with 2 ml of dil .ferric chloride solution.	Red colour developed

4.3.PHYTOCHEMICAL SCREENING – AYAPODI ELAGAM

The preliminary phytochemical screening test was carried out for each extract of Ayapodi Elagam as per the standard procedure at The Tamilnadu Dr.M.G.R.Medical University, Guindy, Chennai 32.[83]

Detection of carbohydrate:

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

a) Molisch's test:

To 2ml of sample extract, two drops of alcoholic solution of α-naphthol were added. The mixture was shaken well and a few drops of concentrated sulphuric acid were added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

b) Benedict's test:

Filtrate were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Detection of glycosides:

Extract were hydrolyzed with dil.HCL and then subjected to test for glycosides. Modified Borntrager's test:

Extract were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonium solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

Detection of flavonoids:

a) Alkaline reagent test:

Extract were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate test:

Extract were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Test for Quinones:

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

The preliminary phytochemical studies of aqueous extract of Ayapodi Elagam were done using standard procedures. The results were presented in table. The present study reveals that the bioactive compounds were present in all the extracts of Ayapodi Elagam.

4.4. THE MICROBIAL LOAD

The Microbial load was done in Regional Research Institute of Unani Medicine, Royapuram, Chennai-600 013.

Analysis of Microbial Load as per WHO,2007[84]

Total viable aerobic count

The total viable aerobic count (TVC) of the herbal material being examined is determined, as specifi ed in the test procedure below, using one of the following methods: membrane-fi ltration, plate count or serial dilution. Aerobic bacteria and fungi (moulds and yeasts) are determined by the TVC.

Usually a maximum permitted level is set for certain products, but when the TVC exceeds this level then it is unnecessary to proceed with determination of specific organisms; the material should be rejected without being subjected to further testing.

Pretreatment of the test herbal material

Depending on the nature of the crude medicinal plant material, grind, dissolve, dilute, suspend or emulsify it using a suitable method and eliminate any antimicrobial properties by dilution, neutralization or filtration. Either phosphate buffer pH 7.2; buffered sodium chloride-peptone solution, pH 7.0; or fluid medium, used for the test, is used to suspend or dilute the test specimen.

Water-soluble materials

Dissolve or dilute 10 g or 10 ml of plant material, unless otherwise specifi ed in the test procedure for the material concerned, in lactose broth or another suitable medium proven to have no antimicrobial activity under the conditions of the test. Adjust the volume to 100 ml with the same medium. (Note that some materials may require the use of larger volumes.) If necessary, adjust the pH of the suspension to about 7.

Test procedures

Plate count

For bacteria:

Use Petri dishes 9–10 cm in diameter. To one dish add a mixture of 1 ml of the pre-treated plant material and about 15 ml of liquefied casein-soybean digest agar at a temperature not exceeding 45 °C. Alternatively, spread the material on the surface of the solidified medium in a Petri dish. If necessary, dilute the material to obtain an expected colony count of not more than 300. Prepare at least two dishes using the same dilution, invert them and incubate them at 30–35 °C for 48–72 hours, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculates the results using the plate with the largest number of colonies, up to a maximum of 300.

For fungi:

Use Petri dishes 9–10 cm in diameter. To one dish add a mixture of 1 ml of the pretreated material and about 15 ml of liquefied Sabouraud glucose agar with antibiotics (also used is potato dextrose agar with antibiotics) at a temperature not exceeding 45 °C. Alternatively, spread the pretreated material on the surface of the solidified medium in a Petri dish. If necessary, dilute the material as described above to obtain an expected colony count of not more than 100. Prepare at least two dishes using the same dilution and incubate them upright at 20–25 °C for 5 days, unless a more reliable count is obtained in a shorter period of time. Count the number of colonies formed and calculates the results using the dish with not more than 100 colonies.

4.5. ANTIBACTERIAL ACTIVITY:

Antibacterial activity was done at M.K.University, Madurai – 625021.

Antibacterial activity of compound towards human pathogenic bacteria was tested by serial dilution method on Luria Bertani (LB) agar plate. The human pathogenic Gram - positive bacteria such as *Bacillus cereus* MTCC 430 and Gram – negative bacteria *Serratiamarcescens* MTCC 4822 were obtained from Microbial Type Culture Collection (MTCC) Chandigarh, India. The above said human pathogens were inoculated in 20mL of sterile Nutrient broth and it was incubated at 37ºC for 12h. After incubation the bacterial culture were treated with compound (200µg/ml) and the culture was incubated for 6 hrs. After the treatment adds100µl of culture test tubes 101 to 107 was diluted and each dilutionwas taken 100 µl of sample spread on LB agar plates using with spreader. The plates were incubated for 24 hours at 37°C. After incubation, the results were observed plates and colonies were counted.

4.6.SPECTROSCOPIC ANALYSIS:

Ayapodi Elagam was analyzed in the presence of heavy metals by using Atomic Absorption Spectrometer(AAS).This study was done at Asthagiri Herbal Research Foundation, 162-A, Perugudi Industrial Estate, Perungudi, Chennai-96.[83]

SEM and EDAX were done at M.K.University, Madurai – 625021.FT-IR was done at IIT Madras, Chennai.

4.6.1. ATOMIC ABSORPTION SPECTROMETER (AAS)

INSTRUMENT DETAILS:

UV-V is spectrometer AA240 series, UV 8500 Absorption Spectrometer (AAS) was used for the analysis. The operating parameters:

Test Method	:	USP 231 USP 39 NF34
Instrument	:	AAS
Model	:	AA240
Standard	:	Sigma standard Fe
Sample preparation	:	For testing Fe- sample in Aqua Regia
Lambda max	:	Fe-248.3 nm

4.6.2. FT-IR:

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time.

The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum. For other uses of this kind of technique, see Fourier transform spectroscopy.

The standard method to prepare a solid sample for FTIR spectrometer is to use KBr. About 2 mg of Ayapodi Elagam and 200 mg KBr are dried and ground. The particle size should be unified and less than two micrometers. Then, the mixture is squeezed to form transparent disc which can be measured directly. For liquids with a high boiling point or viscous solutions, it can be added in between two NACL pellets.

Then the sample is fixed in the cell by skews and measured. For a volatile liquid sample, it is dissolved in CS_2 or CCl_4 to form 10% solution. Then the solutions are injected into a liquid cell for measurement. Gas sample needs to be measured in a gas cell with two KBr windows on each side. That gas cell should first be vacuumed. Then the sample can be introduced into the gas cell for measurement.

4.6.3. SEM:

A SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, crosses sections. The primary electron beam interacts with the sample in a number of key ways:-

- A primary electron generates low energy secondary electron, which tends to emphasize the topographic nature of the specimen.
- A primary electron can be backscattered which produces images with a high degree of atomic number (Z) contrast.
- Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection. The X-ray emitted is characteristic of the elements in the top few µm of the sample.

The SEM is carried out by using FEI-Quanta FEG 200-High Resolution Instrument.

Resolution **:** 1.2 nm gold particle separation on a carbon substrate

Magnification: From a min of 12X to greater than 1, 00,000X

Application **:** To evaluate grain size, particle size distributions, material homogeneity and intermetallic distributions.

4.7. TOXICOLOGICAL EVALUATION OF AYAPODI ELAGAM (A.E)

The following in vivo toxicity studies were carried out on Ayapodi Elagam (A.E) by World Health Organization (WHO) guideline[85.]

Acute Oral Toxicity study (WHO Guideline)

Long term toxicity study (WHO Guideline)

The toxicity studies were carried out at National Institute of Siddha. The study was done after getting permission from the Institutional Animal Ethics Committee.

IAEC Approved No: For acute and long term toxicity study – NIS/IAEC-I/2016/10

For Acute and long term toxicity studies test animals were obtained from Tamil Nadu Veterinary and Animal Sciences University, Madhavaram. Animals were kept in animal house, National Institute of Siddha, Chennai.

4.7.1. DESCRIPTION OF THE METHOD

Selection of the animals:

Animals were selected as per guideline. Healthy adult animals of Wistar albino rat, both male and female rats were used for acute oral toxicity study and Long term toxicity study. The female animals used in the studies were nulliparous and non-pregnant.

Housing and feeding conditions:

Tempereture: In the experimental animal room: 22ºC (± 3ºC)

Humidity: 60 ± 10 %

Lighting : Artificial, the sequence being 12 hours light, 12 hours dark.

The animals were housed in polypropylene cages provided with bedding of husk. The animals had free access to RO water. For feeding, Standard pellet diet (bought from SaiMeera foods pvt. Ltd, Bangalore) was used.

Preparation of animals:

The animals were randomly selected, to permit individual identification by cage number and individual marking on the fur of each animal was made with picric acid. The animals were kept in their cages for 7 days prior to dosing to allow for acclimatization to the laboratory conditions. The principles of laboratory animal care were followed.

Test Substance:

Ayapodi Elagam (A.E) was black in colour, without taste and odour. The drug was dissolved in saline to obtain and ensure the uniformity in drug distribution.

Route of administration:

Oral route was selected, because it is the normal route of clinical administration.

Preparation of doses:

The stock solution was prepared freshly as dose per animal suspended in 1ml saline.

PROCEDURE:

4.7.2. ACUTE ORAL TOXICITY STUDY

Test Animals:

Species and strain	:	Wistar Albino rat
Sex	:	Male and Female
Age, Weight	:	6-8 weeks, 160-180 g
Test guideline	:	WHO guideline
Groups/treatment	:	Grouped by randomization
Duration of exposure to the “Ayapodi Elagam”(A.E)	:	Single dose
Study duration	:	14 days
Number of animals	:	10 male, 10 female / group
Route of administration	:	Oral

Number of animals and dose levels:

Animals were divided into two groups each group contains 5 male and 5 female rats.One group as control and the other as test group. Control group was treated with saline and other group was treated with test drug Ayapodi Elagam (A.E) ten times more then the therapeutic dose (5000mg per kg b.wt).

No of animals used in Acute toxicity study:

Groups	No. of Rats
Group I : Vehicle control (saline)	5 male, 5 female
Group II : Test drug (5000mg / kg b.wt)	5 male, 5 female

Administration of doses:

The test drug was administered in a single dose by using oral gavage. Animals were fasted prior to drug administration. Following the period of fasting, the animals were weighed and test drug was administered. The control groups received equal volume saline. The test drug was administered at 10 times the therapeutic dose (5000 mg / kg b.wt). The food was withheld for 3-4 hours after dosing the animal.

Observations:

Observations were made and recorded systematically and continuously observed after the drug administration as per the guideline.

- ½ hour, 1 hour, 2 hours, 4 hours and up to 24 hours observation.
- All rats were observed twice daily for further 14 days.
- Body weight were Calculated weekly once.
- Feed and water intake were Calculated daily.

Cage side observation

The animals were monitored for behavioral parameters like Alertness, Aggressiveness, Piloerection, Grooming, Gripping, TouchResponse, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, and Mortality.

Gross necropsy:

At the end of the 14th day all the animals were sacrificed by using the injection of Pentothal sodium Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. The brain,eye, lungs, heart, spleen, liver, kidneys, adrenaland sex organs of all animals.

4.7.3. LONG TERM TOXICITY STUDY OF AYAPODI ELAGAM[85]

Test animals:

Species and strain	:	Wistar albino rats
Sex	:	Male and Female
Age, Weight	:	6 -8 weeks, 160-180 g
Test guideline	:	WHO guideline
Groups/treatment	:	Grouped by randomization
Duration	:	90 days
Number of animals	:	10/group (5/sex)
Route of administration	:	Oral

Grouping of animals:

Long term toxicity study was carried out at different dose levels. The animals in both sex was divided in four groups (group I, II, III & IV). Each group consists of 10 animals (5 males and 5 females).

Group-I served as control and the other three groups II, III and IV for test drug of Low dose (450mg/kg/b.wt), Mid dose(900mg/kg/b.wt) and High dose(1800mg/kg b.wt) respectively[The low dose was calculated from the therapeutic dose (5g) and body surface area of rat (0.018). Calculation of low dose – 5000 × 0.018 = 90 mg/200 g of animal]

Low dose = 90 × 5 = 450 mg/kgb.wt
Mid dose = low dose × 2
= 450 × 2 = 900 mg/kg b.wt
High dose = low dose × 4
High dose = 450 × 4 = 1800 mg/kg b.wt

No of animals used for long term toxicity study:

Groups	No of Rats
Group I : Vehicle control (Saline)	10 (5male, 5female)
Group II : (Test drug) Low dose (450 mg/kg b.wt)	10 (5male, 5female)
Group III : (Test drug) Mid dose (900 mg/kg b.wt)	10 (5male, 5female)
Group IV : (Test drug) High dose (1800 mg/kg b.wt)	10 (5male, 5female)

Administration of doses:

The animals were dosed with the test drug daily for a period of 90 days. The test drug mixed with saline and was administeredby oral gavage, and this was done in a single dose to the animals once in daily for 90 days.

Observations:

Experimental animals were kept under observation throughout the course of study for the following

- All rats were observed twice daily for 90 days.
- Body weight were Calculated weekly once.
- Feed and water intake were Calculated daily.

Cage side observation

The animals were monitored for behavioral parameters like, Alertness, Aggressiveness, Piloerection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, and Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, and Mortality.

Laboratory Investigations:

On the 91st day, the animals were fasted overnight, then anesthetized to collect blood samples from the abdominal aorta in two tubes: one with EDTA for hematological parameters, another one without any anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum for biochemical parameters.

Hematological Investigations:

Blood samples of control and experimental rats were analyzed for Haemoglobin (Hb), total Red Blood Corpuscles (RBC), Differential Count (DC), White Blood Corpuscles (WBC) count, Packed Cell Volume(PCV), Platelet, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH),Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated by auto analyzer.

Biochemical Investigations:

Serum samples of control and experimental animals were analyzed for, Bilirubin, BUN, Creatinine, Triglyceride, Total Cholesterol, HDL, LDL, VLDL, using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (SGOT/AST), glutamate pyruvate transaminase/ Alanine aminotransferase (SGPT/ALT) were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on the 91st day except the high dose group four animals. The high dose group two male and two female animals were set as satellite group. The satellite group was sacrificed on after 30 days. Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, eye, lung, heart, spleen, liver, kidney, adrenal,sex organsand bone marrow of all animals were collected and sent for histopathological evaluation.

Histopathology:

The organs included liver, kidney, spleen, brain, heart, lung, stomach and bone marrow of the animals were preserved, and they were subjected to histopathological examination.

The organ pieces (35µm thick) of all the animals (control, high dose and satellite group) were preserved and fixed in 10% formalin for 24 hrs. Samples were dehydrated in an auto technic and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50^{o}C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were Prepared then stained with Haematoxylin-eosin.

Bone Marrow Smear:

About 0.2 ml of smear aspirated from the femur thigh bone of the experimental animal was dropped onto the glass slide and made in to thin smear allow the smear to dry. Dried smear was stained with leishman stain and washed. Followed by this cedar wood oil was placed on to the smear and was observed microscopically.[86]

Statistical analysis:

Findings such as body weight changes, food consumption, water intake, heamatology and biochemical analysis were subjected to One-way ANOVA Dennett's test using a computer software program followed by Graph Pad Instat-3.[87]

RESULTS

5.1. QUALITATIVE ANALYSIS

A.PHYSICO-CHEMICAL ANALYSIS

Table-1: Colour, Odour and nature of Ayapodi Elagam

S.No	Parameters	Results
1	Colour	Black
2	Odour	Odourless
3	Solubility	• Soluble in water & milk • Insoluble in acetone& ether
4	Nature	Semisolid
5.	pH at 25°C(1%w/v solution)	4.65

From Table 1, The Organoleptic characters shows that Ayapodi Elagam is Black in colour and Odourless semisolid form of drug. It is soluble in water, milk and insoluble in acetone and ether and pH is acidic in nature.

Table-2: Physico-chemical properties of Ayapodi Elagam

S.NO	Parameters	Percentage
1	Loss on drying	5.23%
2	Total ash value	47.93%
3	Acid insoluble ash	42.96%
4	Water soluble ash	4.58%
5	Water soluble extraction	37.18%
6	Alcohol soluble extraction	14.1%

From Table 2, The Physico-chemical analysis of Ayapodi Elagam explained in the parameters such as Moisture content, Total ash value, Acid insoluble ash, Water soluble ash, Water soluble extraction and Alcohol soluble extraction were within the normal limits.

B.BIO-CHEMICAL ANALYSIS:

Table-3: Test for Basic radicals on Ayapodi Elagam

S.No	Procedures	Ayapodi Elagam
1	Test for Ammonium	+
2	Test for Sodium	-
3	Test for Magnesium	+
4	Test for Aluminium	+
5	Test for Potassium	-
6	Test for Calcium	+
7	Test for Ferrous iron	+
8	Test for Copper	-
9	Test for Zinc	+
10	Test for Arsenic	-
11	Test for Mercury	-
12	Test for Lead	-

(+ve /- ve present or absent if component tested)

From Table 3, The Biochemical analysis for basic radical reveals that Ayapodi Elagam contains Ammonium, Magnesium, Aluminium, Calcium, Iron, and Zinc.

Table-4: Test for Acidic radicals on Ayapodi Elagam

S.No	Procedures	Ayapodi Elagam
1	Test for Sulphate	-
2	Test for Chloride	-
3	Test for Phosphate	+
4	Test for Flouride&Oxalate	-
5	Test for Nitrate	-
6	Test For Carbonate	+
7	Test For Sulphide	-
8	Test For Borate	-
9	Test For Nitrite	-

(+ ve /- ve present or absent if component tested)

From Table 4, The Biochemical analysis for acid radicals reveals that Ayapodi Elagam contains Phosphate and Carbonate.

Table-5: Test for Miscellaneous on Ayapodi elagam

S.No	Procedures	Ayapodi Elagam
1	Test for Starch	-
2	Test for Reducing sugar	+
3	Test for Alkaloids	+
4	Test for Amino Acids	-
5	Test for Tannic acids	-
6	Test For Unsaturated Compound	-
7	Test for type of compounds	Anti pyrine, Aliphatic amino acids and meconic acid are present

(+ ve /- ve present or absent if component tested)

From Table 5, The Biochemical analysis for Miscellaneous reveals that Ayapodi Elagam contains Reducing sugar,Alkaloids, Anti pyrine, Aliphatic amino acids and Meconic acid are present.

C.PHYTOCHEMICAL ANALYSIS:

Table 6, Test for phytochemical screening on Ayapodi Elagam

S.No	Phytochemicals	Test name	H_2O ext.
1	Alkaloids	Mayer's Test	-ve
		Wagner's Test	-ve
2	Carbohydrates	Molisch's Test	+ve
		Benedict's Test	+ve
3	Glycosides	Modified Borntrager's Test	+ve
		Keller killian Test	-ve
4	Saponin	Froth Test	-ve
		Foam Test	-ve
5	Phytosterol	Salkowski's Test	-ve
6	Phenols	Ferric Cholride Test	-ve
7	Tannins	Gelatin Test	-ve
8	Flavonoids	Alkaline reagent Test	+ve
		Lead acetate Test	+ve
9	Proteins & amino acids	Xanthoproteic Test	-ve
10	Diterpenes	Copper acetate Test	-ve
11	Gum &Mucilage	Extract + alcohol	-ve
12	Fixed oil &Fat	Spot Test	-ve
13	Quinones	NAOH + Extarct	+ve

(+ ve /- ve present or absent if component tested)

From the table 6, phytochemical screening shows presence of Carbohydrates,Glycosides, Flavonoids and Quinones.

D. MICROBIAL LOAD FOR AYAPODI ELAGAM

Table 7, Microbial load for Ayapodi Elagam

S.No	Parameters	Result	WHO Permissible Limits (Internal use)
1	Total Bacterial Count (TBC)	Less than 1 cfu/gram	Maximum 10^5 per/g
2	Total Fungal Count(TFC)	Less than 1 cfu/gram	Maximum 10^3per/g
3	*Enterobacteriaceae*	Absent	Maximum 10^3per/g
4	*Escherichia coli*	Absent	Maximum 10per/g
5	*Salmonella Spp*	Absent	None
6	*Staphylococcus aureus*	Absent	None
7	*Pseudomonas aeruginosa*	Absent	None

From the Table 7, Microbial Load for Ayapodi Elagam the Total Bacterial Count and Total Fungal Count was with in the limit.*Enterobacteriaceae, Escherichia coli,Salmonella Spp,Staphylococcus aureus, P.seudomonas aeruginosa*were absent as per WHO guideline.

E. ANTI-BACTERIAL ACTIVITY:

The compound against *S.marcescens* MTCC 4822 showed the bacterium uncountable colonies (Too Numerous To Count (TNTC)) was grown on LB agar plates compared with control. *B. cereus* MTCC 430 towards the compound, bacterial colonies reduced in each dilution and colony was counted.

Figure 1: Colonies growth on LB agar plates in various dilutions

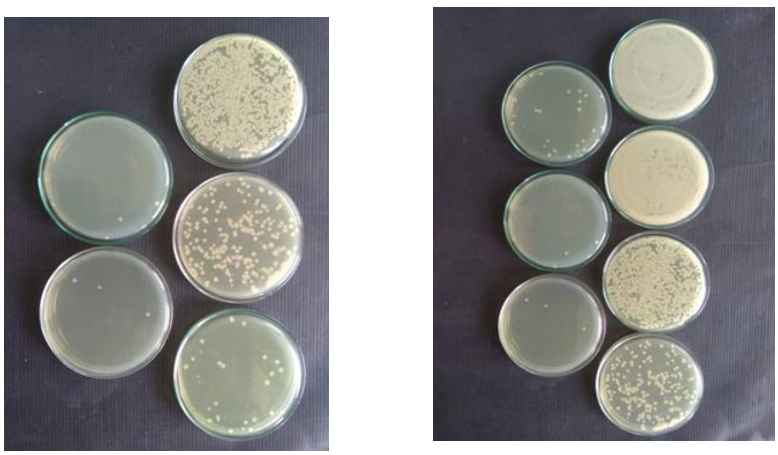


Table 8, Anti-bacterial activity of Ayapodi Elagam

S. No.	Human Pathogens	10^1	10^2	10^3	10^4	10^5	10^6	10^7
1.	*Sterratia marcescens*	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
2.	*Bacillus cereus*	TNTC	TNTC	TNTC	162	29	5	3

TNTC – Too Numerous To Count

From the Table 8, The Ayapodi Elagam has anti-bacterial activity against *B.cereus.*

5.2. SPECTROSCOPIC ANALYSIS:

A. ATOMIC ABSORPTION SPECTROSCOPY

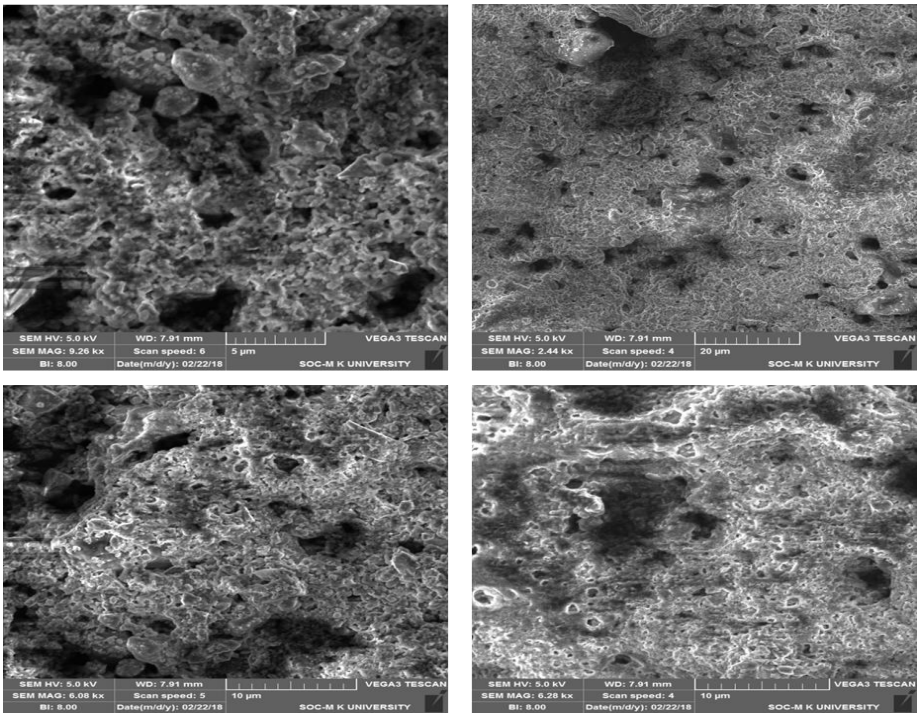
Iron content of Ayapodi Elagam was analyzed by AAS this result is

Fe content in the sample --- 12.94%

B. SCANNED ELECTRON MICROSCOPY

Determination of Particle size onAyapodi Elagam

Figure 2. SEM image of Ayapodi Elagam

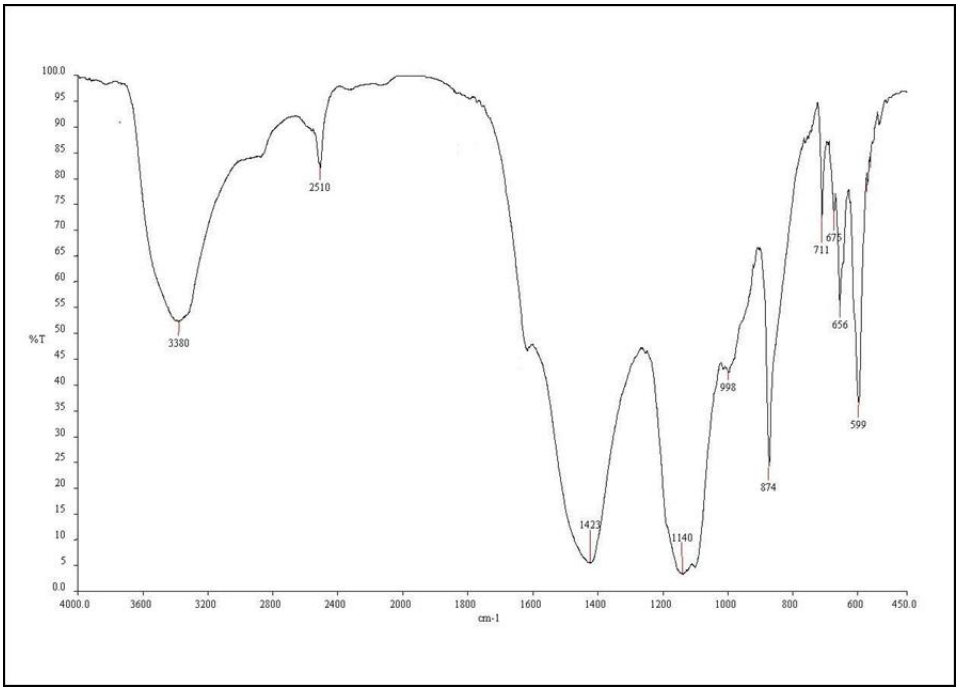


Results:

Particle Size	:	Micro size
Shape	:	Irregular
Surface	:	Smooth
Distribution	:	Aggregate

C. FT-IR Analysis of Ayapodi Elagam

Graph 1 – FT-IR graph of Ayapodi Elagam

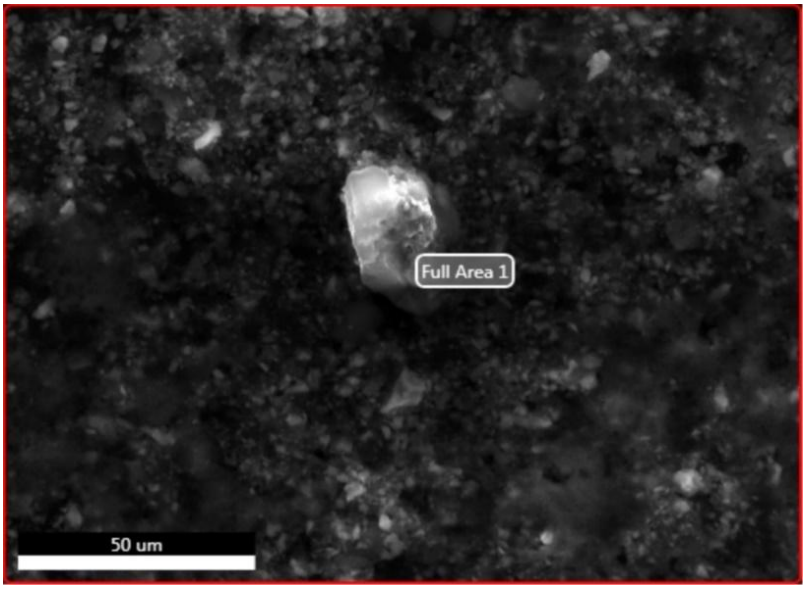


Graph 1. Shows the FTIR spectrum of the Ayapodi Elagam sample and its functional groups. Before transfer, the bands from 3020 to 2952 cm−1 are ascribed to the stretching vibrations of the C–H bond of organic compounds. The band at 1140 cm−1 is assigned to the stretching vibration of the C–O bond of oleic acid. The band at 1423 cm−1 can be ascribed to the asymmetric and symmetric stretches of COO–, indicating that the acid chain is attached to the Fe_3O_4. The strong absorption band at 599 cm−1 is assigned to Fe–O vibrations of Fe_3O_4. There is a slight decrease in the bands intensity at 2510 cm−1, suggesting the reservation of N-H chains on the surface of Fe_3O_4. A band at 3437 cm−1 that corresponds to the vibration of O–H is enhanced due to absorbed water on the surface after transfer.

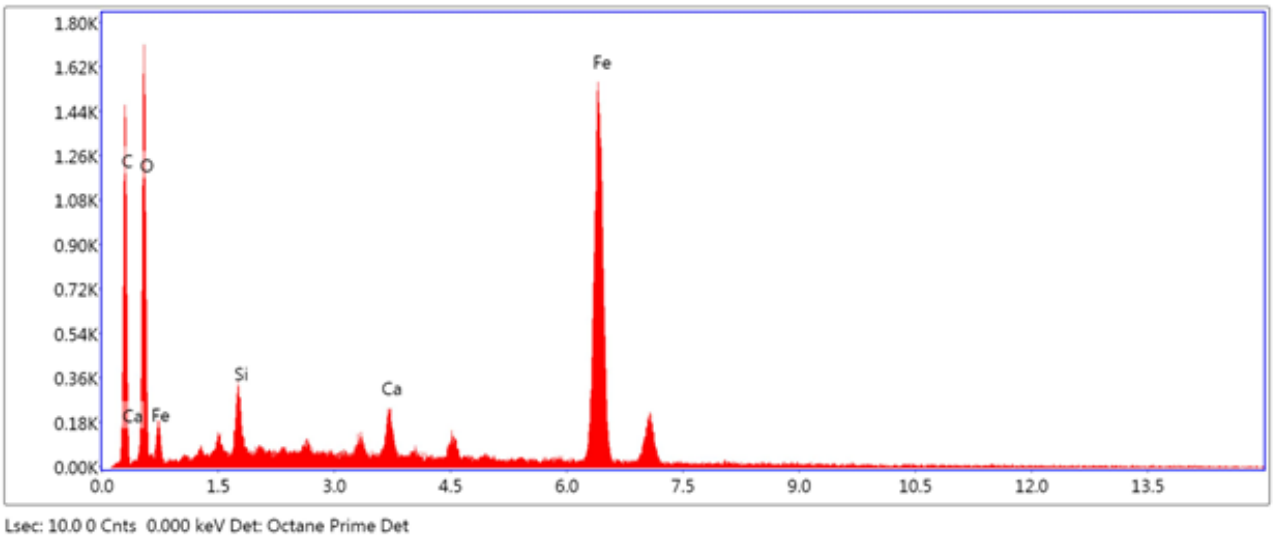
D. EDAX For Ayapodi Elagam:

Figure 3. EDAX image for Ayapodi Elagam

Area 485



Graph 2 – EDAX graph for Ayapodi Elagam



EDAX analysis for Ayapodi Elagam Sample

Element	Weight %	Atomic %	Net Int.	Error %
C K	20.47	46.05	661.48	10.27
O K	12.06	20.36	892.55	8.63
SiK	1.19	1.15	190.61	10.60
CaK	2.04	1.38	203.40	8.91
FeK	64.23	31.07	2065.05	3.39

EDAX analysis shows the elements present in the sample as shown in fig 3& table 9, Represents the weight and atomic percentage of sample. The presence of iron is 64 Wt% because using magnetic separation during sample preparation.The presence of Si and Ca is very small contribution by the presence of sand during sample collection and the herbal drugs Nellikai (*Phyllanthus emblica*), Keezhkainelli (*Phyllanthus nirui*), Karisalai*(Eclipta alba*) in the sample.

5.3.ACUTE TOXICITY STUDY

Table 10, Behavioural Signs of Acute Toxicity Study of Ayapodi Elagam

Parameters	30 mints		4 hrs		24 hrs		1st week		2nd week	
	C	T	C	T	C	T	C	T	C	T
Skin & Fur	N	N	N	N	N	N	N	N	N	N
Mucous Membrane	N	N	N	N	N	N	N	N	N	N
Respiratory rate	N	N	N	N	N	N	N	N	N	N
Heart rate	N	N	N	N	N	N	N	N	N	N
Salivation & Lacrimation	N	N	N	N	N	N	N	N	N	N
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Piloerection	N	N	N	N	N	N	N	N	N	N
Urinary incontinence	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Defecation	N	N	N	N	N	N	N	N	N	N
Sleep & Gait	N	N	N	N	N	N	N	N	N	N
Tremors &Convulsion	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

N – Normal, C – control, T – Test group.

All the data were summarized in the form of (Table-10) revealed that there was no abnormal signs and behavioural changes in all animals at the dose level of 5,000 mg/kg body weight administered orally during the study period.

There was no mortality observed after dosing of Ayapodi Elagam upto 5000mg/kg body weight during the study period of 14 days. This indicates that the LD50 of Ayapodi Elagam is more than 5000mg/kg b.wt.

There were no changes in skin and fur, eyes and mucous membranes of all animals. The eating, drinking habit, sleep pattern, locomotion were normal in all animals and no changes in body weight as compared to control group.

At the end of the 14 the day necropsy was performed and there was no abnormality seen in test groups as compared to control group during the examination.

LONG TERM ORAL TOXICITY STUDY

5.4.1. FOOD INTAKE OF ALBINO RATS EXPOSED TO AYAPODI ELAGAM

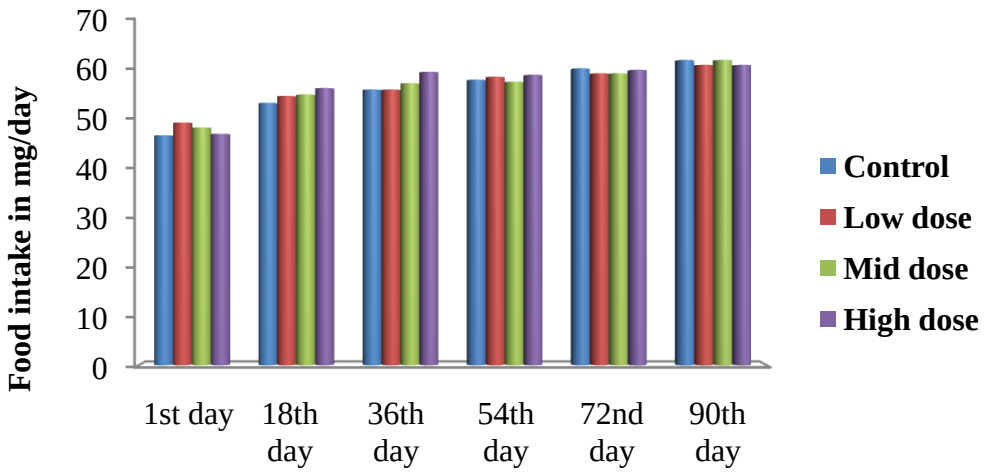
Food consumption ofthe animals no significant difference in food intake the test group animals were observed when compared with control group during the study period. (Table 11) but they were within physiological limit.

Table 11, Food (mg/day) intake of albino rats exposed to Ayapodi Elagam

Dose (mg/ day)	Control	Low dose	Mid dose	High dose
Ist day	46.7±10.4	49.3±19	48.3±12.6	47±10.4
18th Day	53.3±12	54.7±10.01	55±5.0	56.3±12.4
36th day	56±15.6	56±11.53	57.3±11.23	59.6±10
54th day	58±15.14	58.6±13.3	57.6±13.7	59±15.7
72nd day	60.3±13.3	59.3±15.8	59.3±13.3	60±13.2
90th day	62±15	61±13.9	62±11	61±12.3

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01.

Figure 4: Food (mg/day) intake of albino rats exposed to Ayapodi Elagam



5.4.2.WATER INTAKE OF ALBINO RATS EXPOSED TO AYAPODI ELAGAM

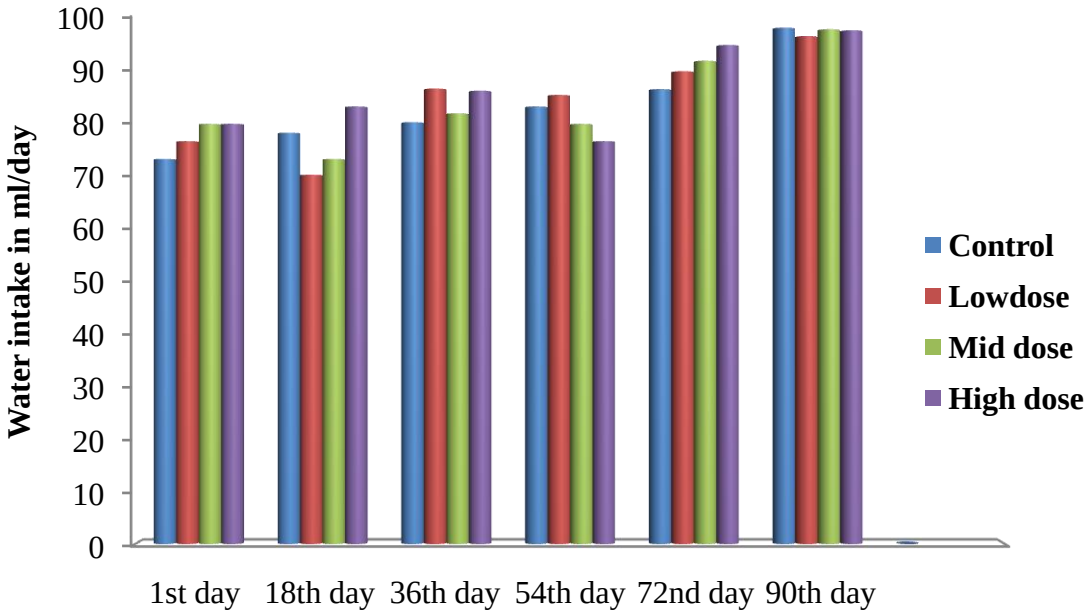
Water consumption of both control and test groups animal shows normal physiological limit throughout the study period.

Table 12, Water (ml/day) intake of albino rats exposed to Ayapodi Elagam

Dose (ml/day)	Control	Low dose	Mid dose	High dose
Ist day	73.3±30.5	76.7±25.16	80±26.5	80±17.32
18th Day	78.3±20.8	70.3±15.3	73.3±508	83.3±15.3
36th day	80.3±15.3	86.7±11.54	82±20	86.3±20.8
54th day	83.3±15.3	85.5±11	80±12	76.7±25
72nd day	86.6±11.54	90±10	92±10	95±15.3
90 th day	98.3±7.6	96.7±11.5	98±5.7	97.8±7.6

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01.

Figure 5: Water (ml/day) intake of of albino rats exposed to Ayapodi Elagam



5.4.3.BODY WEIGHT CHANGES OF ALBINO RATS EXPOSED TO AYAPODI ELAGAM

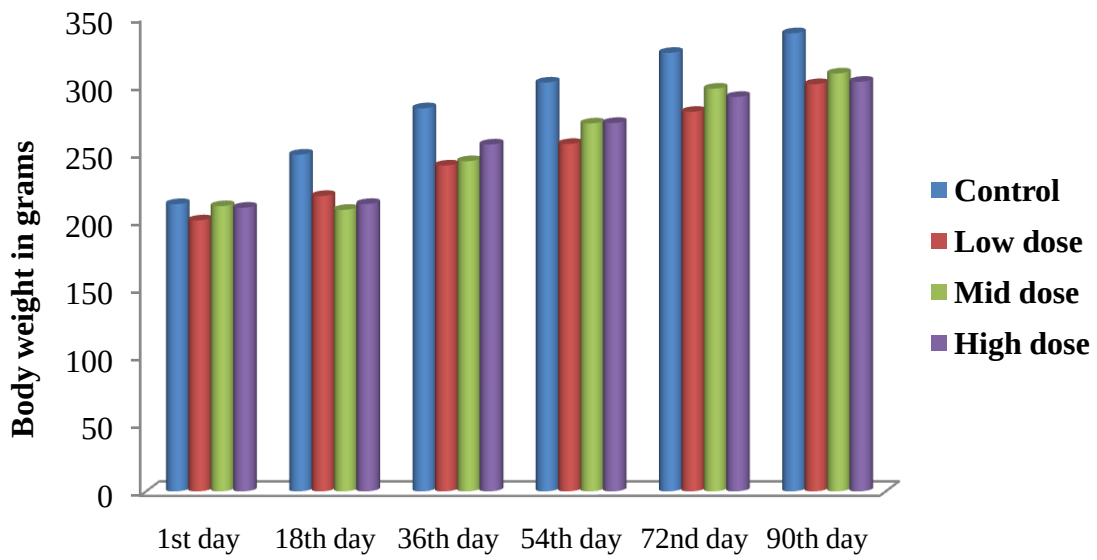
Body weight of both control and test dose group exhibited normal body weight throughout the study period. (Table 13)

Table 13, Body weight (g) changes of albino rats (Male and Female) exposed to AyapodiElagam

Body weight	Ist day	18th Day	36th day	54th day	72nd day	90th day
Control	212±20.7	248.5±20.7	282.7±40	301.7±54	323.6±64.7	338.1±62
Low dose	200±40.8*	218±41.1	240.5±60	256.4±70.3	280.1±77.4	300.5±78
Mid dose	210.5±42.8	207.8±43.0	243.7±69.6	271.5±74.8	297.2±72.7	308.4±74
High dose	209.3±29.6	212.1±29.7	256±50.65	271.9±60.2	291.1±73.9	302.3±74

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01.

Figure 6: Body weight (g) changes of albino rats (male and female) exposed to Ayapodi Elagam



5.4.4. HAEMATOLOGICAL CHANGES OF ALBINO RATS EXPOSED TO AYAPODI ELAGAM

The results of the Haematological investigations conducted at the end of the study the groups revealed slightly significant changes in levels of haematological parameters when compared with control group and post retrieval group haematological parameters towards normal when compared with control group. (Table 14)

Table 14, Effect ofAyapodi Elagam on Haematological Parameters

Parameters	Control	Low dose	Mid dose	High dose	Satellite group
RBC ($\times 10^6$µl)	7.4±0.4	7.6±0.30	7.43±1.34	7.13±1.18	6.6±1.05
WBC ($\times 10^3$µl)	9.2 ±2.05	8.92±1.2	8.89±2.16	9.15±2.17	7.9±0.9
PLT ($\times 10^3$µl)	797±108.3	725.±127.3	757.1±152.6	628.83±100.3	735.5±107.2
HGB (g/dl)	13.58±2.32	13.48±2.01	13.4±1.51	12.61±1.9	14.9±2.3
Neutrophils ($10^3/mm^3$)	1.74±0.54	1.92±0.7	1.75±0.45	1.58±0.3	1.72±0.4
Lymphocyte (%)	73.25±9.08	88.74±10.86**	78.88±8.55	89.35±7.34**	74.8±9.8
Monocyte (%)	3.39±1.7	3.64±1.6	2.4±1.21	3.68±1.49	3.52±0.7
Eosinophil's (%)	1.32±0.26	1.35±0.33	1.36±0.3	1.45±0.23	1.6±0.35
Basophils (%)	-	1	1	-	0
MCH (pg)	20.05±3.01	17.93±3.8	18.65±4.2	19.23±3.70	17.85±3.15
MCV (fl)	58.84±5.13	56.43±5.75	57.96±5.4	59.01±5.03	56.9±4.9

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure 7:Effect of Ayapodi Elagam on RBC

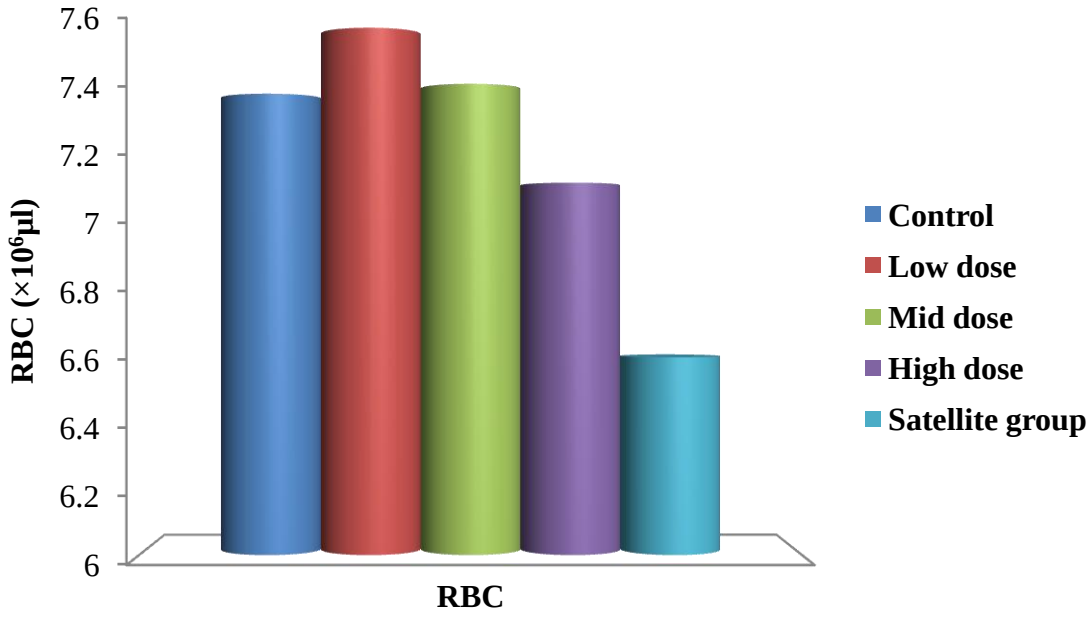


Figure 8:Effect of Ayapodi Elagam on WBC

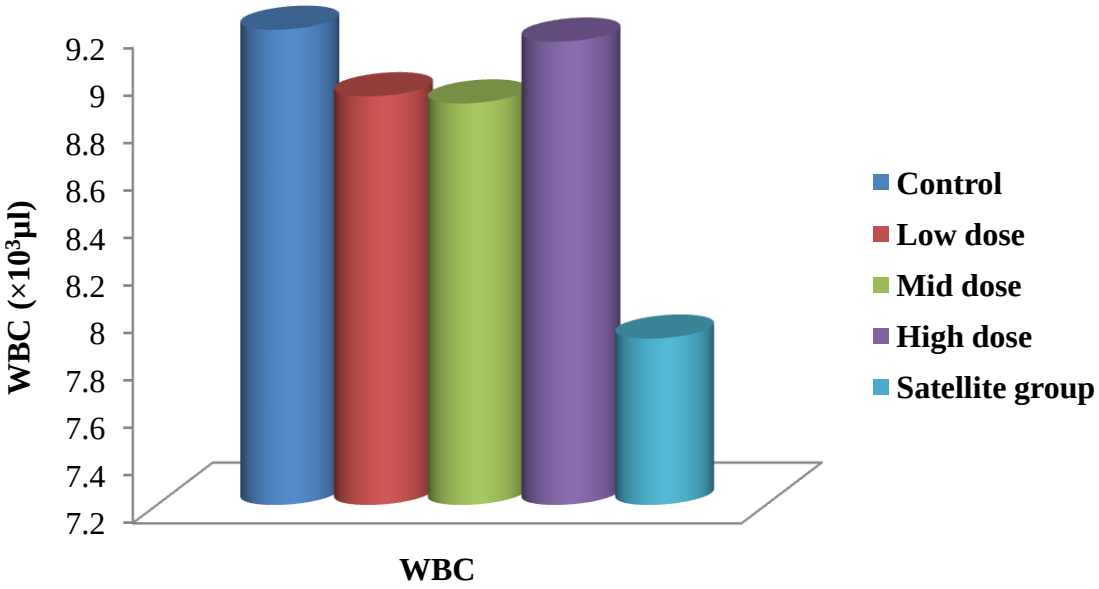


Figure 9:Effect of Ayapodi Elagam on Platelet count

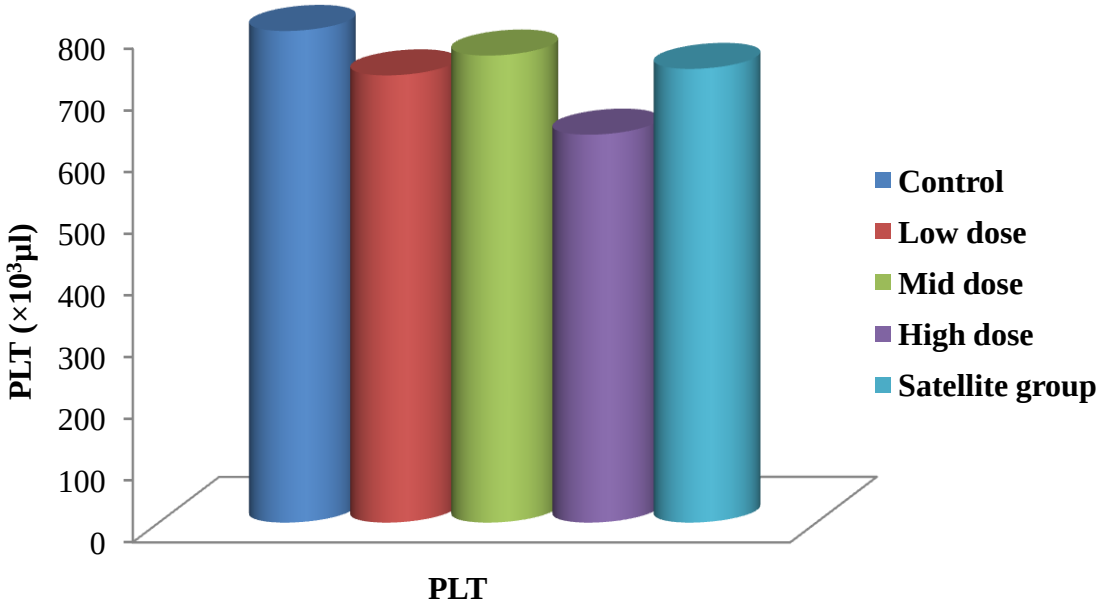


Figure 10: Effect of Ayapodi Elagam on Hemoglobin

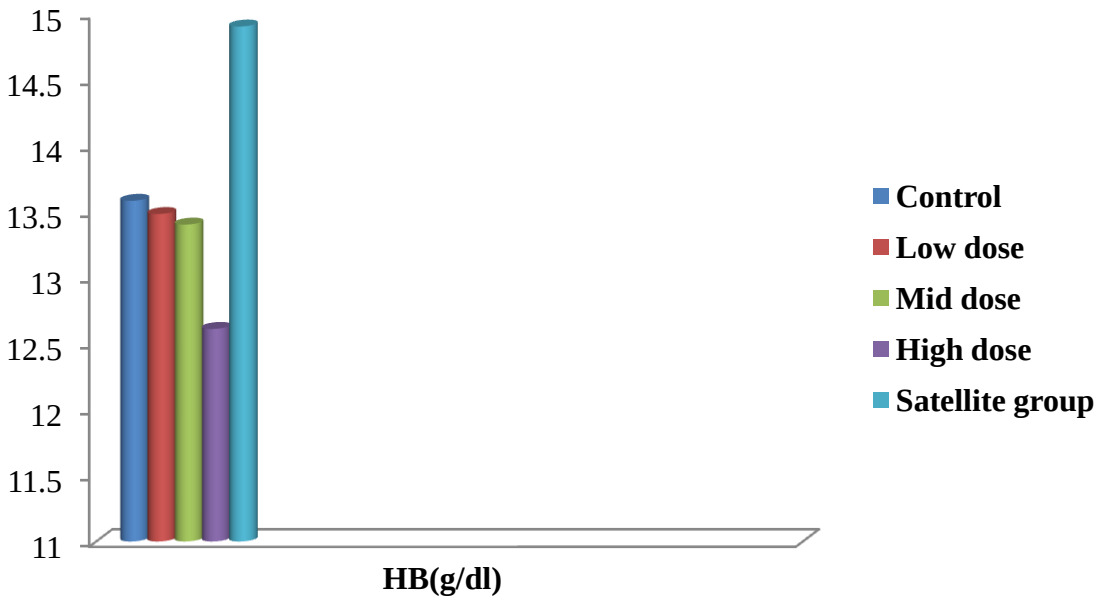


Figure 11: Effect of Ayapodi Elagam on Leucocytes

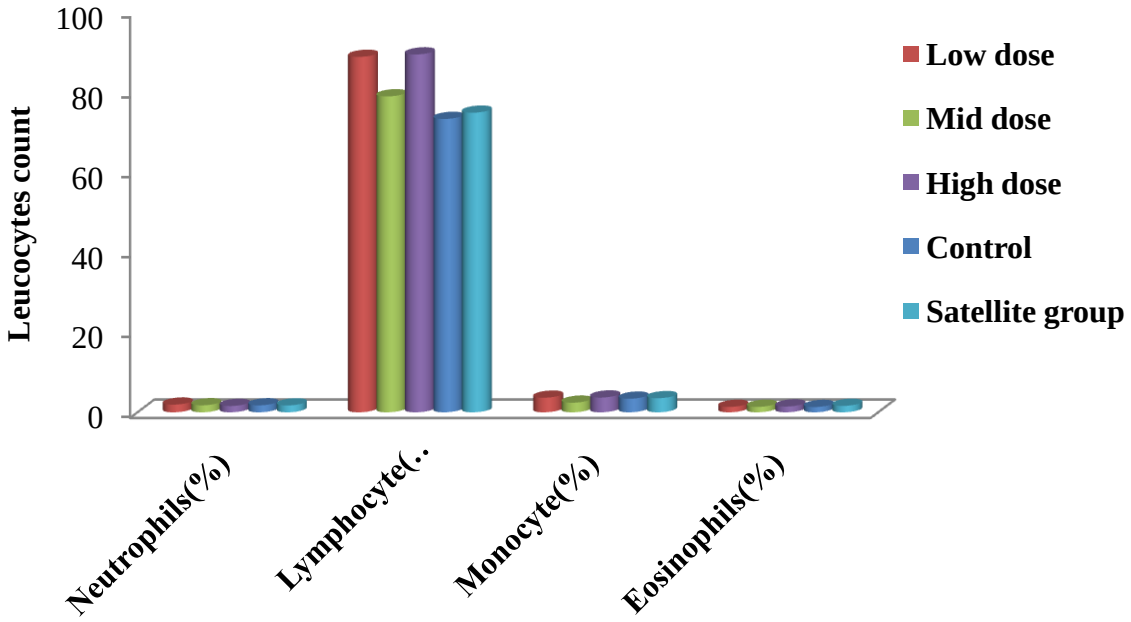


Figure 12: Effect of Ayapodi Elagam on MCH

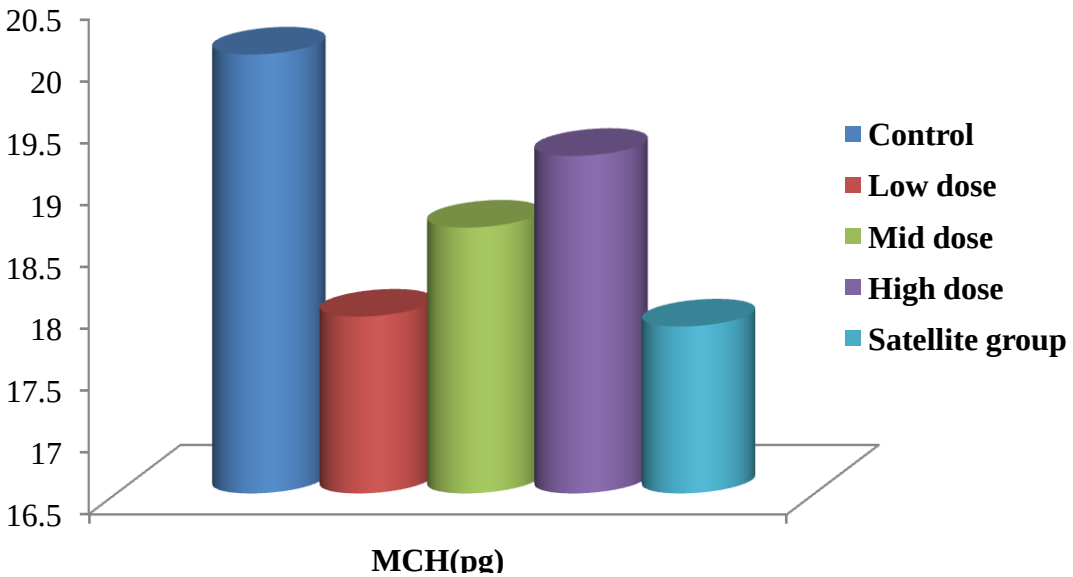
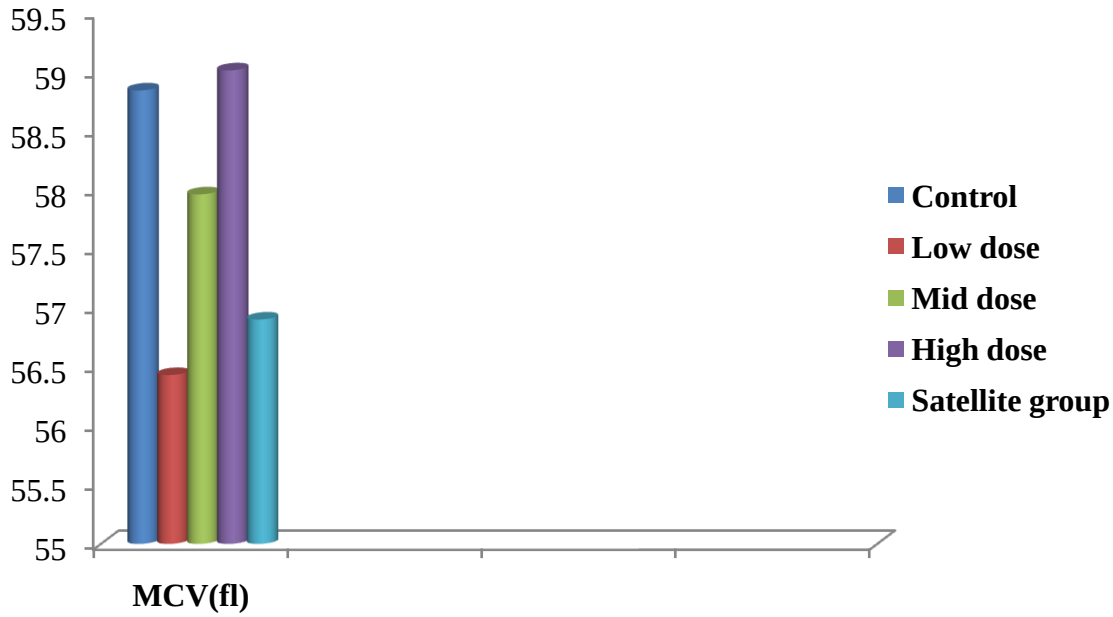


Figure 13: Effect of Ayapodi Elagam on MCV



5.4.5. LIPID PROFILE ON AYAPODI ELAGAM

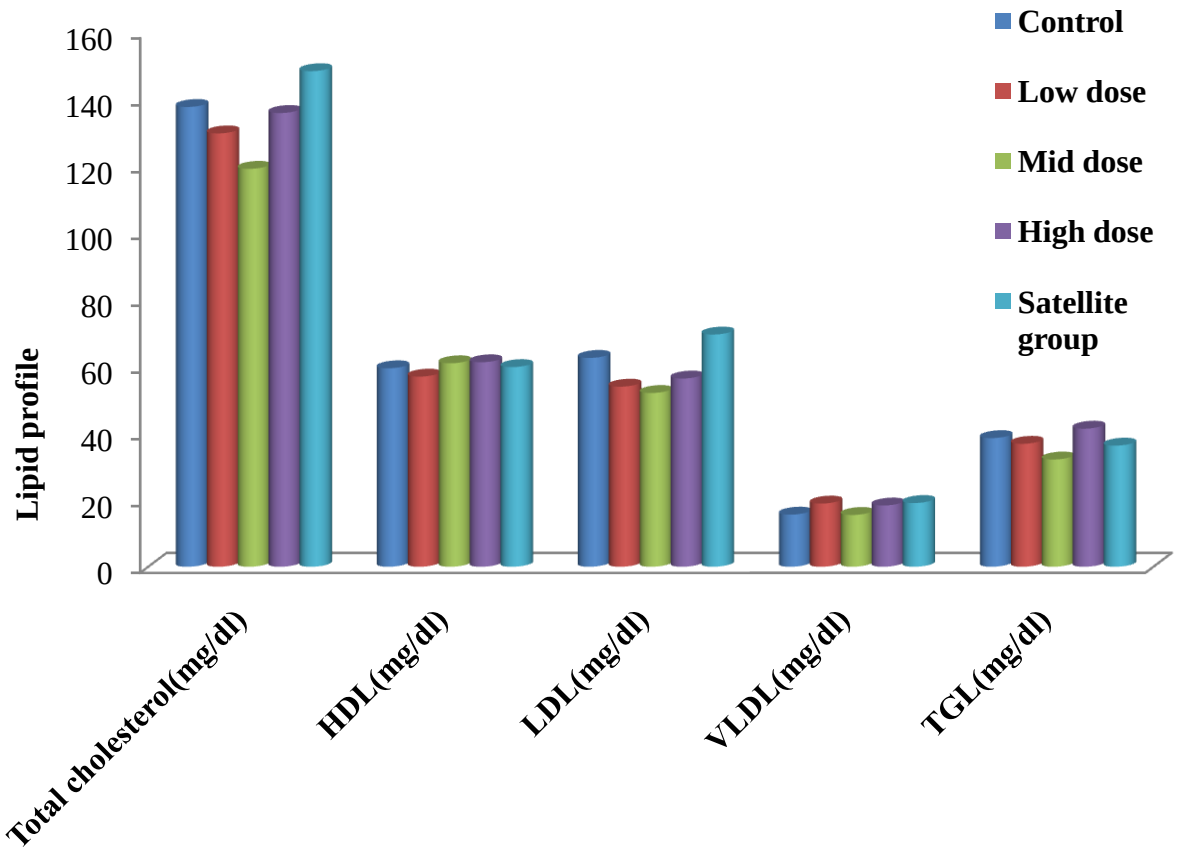
Biochemical investigations were conducted at the end of the study and the results were recorded. In mid dose group showed significant changes present in biochemical parameters when compared with the control group. But the other group the values were in normal physiological limits.

Table 15, Effect of Ayapodi Elagam on Biochemical parameters

Dose (mg/kg)	Control	Low dose	Mid dose	High dose	Satellite group
Total cholesterol (mg/dl)	137.6±14.20	129.7±10.9	119.09±14.70*	135.8±19.4	148.32±19.45
HDL (mg/dl)	59.4±8.53	56.9±5.30	60.9±8.3	61.2±8.08	59.75±8.6
LDL (mg/dl)	62.5±9.64	53.9±9.70	42.7±11.6**	56.33±15.6	69.5±21.64
VLDL (mg/dl)	15.6±2.50	18.91±4.93	15.5±3.5	18.33±3.7	19.07±2.14
Triglycerides (mg/dl)	38.5±10.34	36.8±8.40	32.1±9.3	41.33±8.9	36.25±3.8

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01.

Figure 14: Effect of Ayapodi Elagam on Biochemical parameters



5.4.6. RENAL PARAMETERS ON AYAPODI ELAGAM

The results of the renal functions test conducted at the end of study test groups does not have any significant changes in levels of renal parameters when compared with control group and post retrieval group renal function parameters towards normal when compared with control group.

Table 16, Effect of Ayapodi Elagam on Renal Parameters

Dose(mg/kg)	Control	Low dose	Mid dose	High dose	Satellite group
BUN(mg/dl)	15.9±3.01	15.7±4.8	15.8±5.0	14.33±6.74	16.2±3.9
Creatinine (mg/dl)	0.7±0.12	0.8±0.11	0.7±0.2	0.8±0.15	0.75±0.21

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure15: Effect of Ayapodi Elagam on Renal parameters

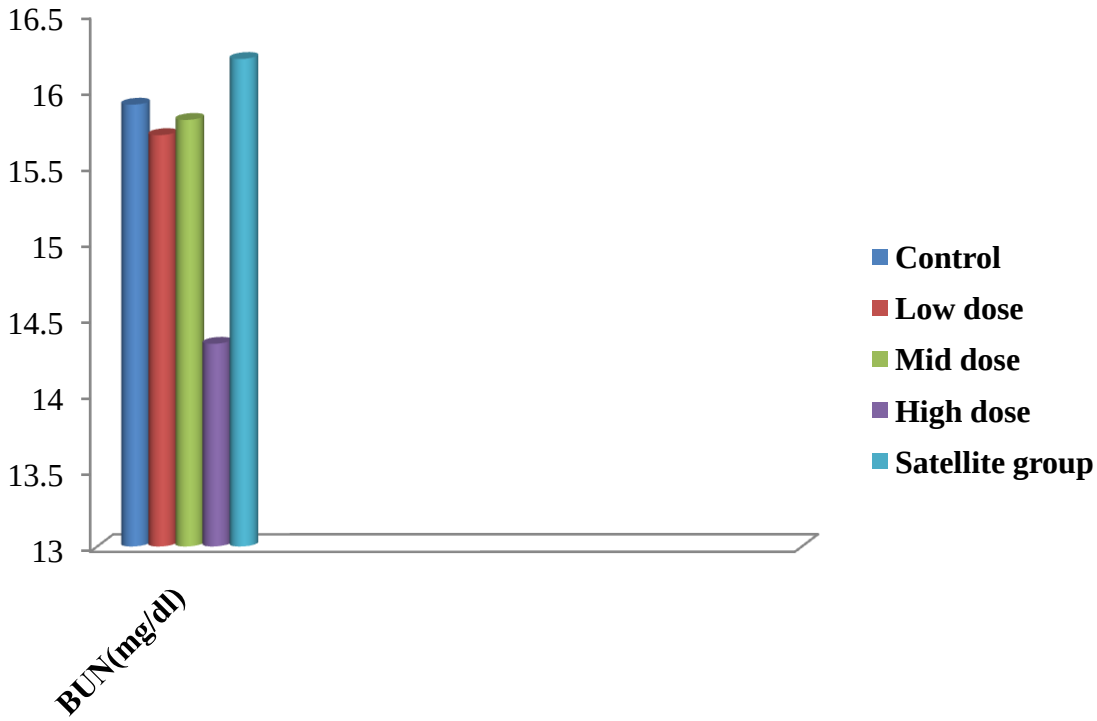
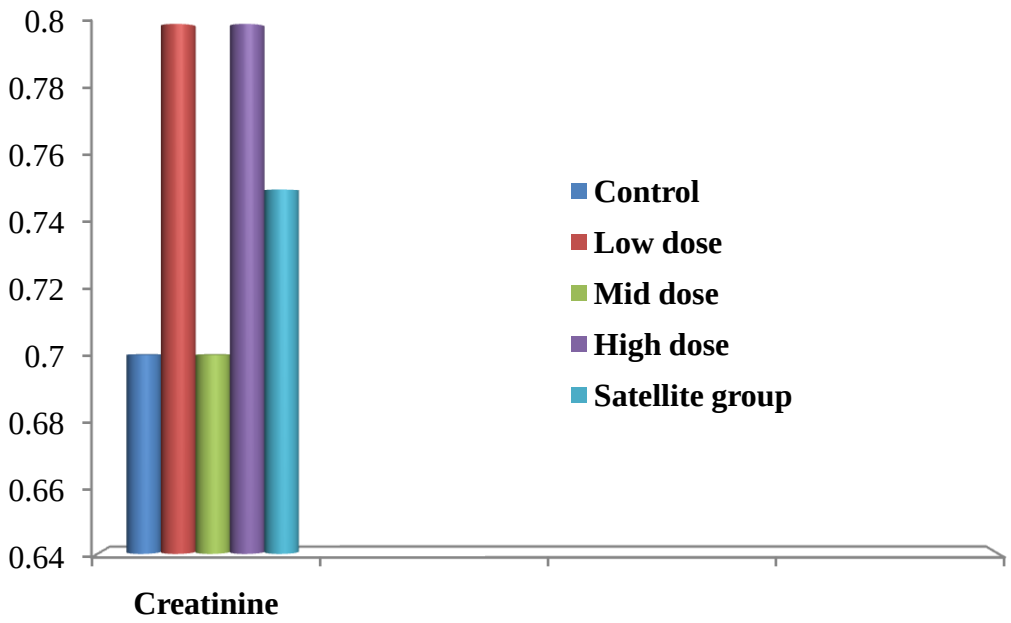


Figure 16.Effect of Ayapodi Elagam on Creatinine



5.4.7. HEPATIC PARAMETER OF AYAPODI ELAGAM

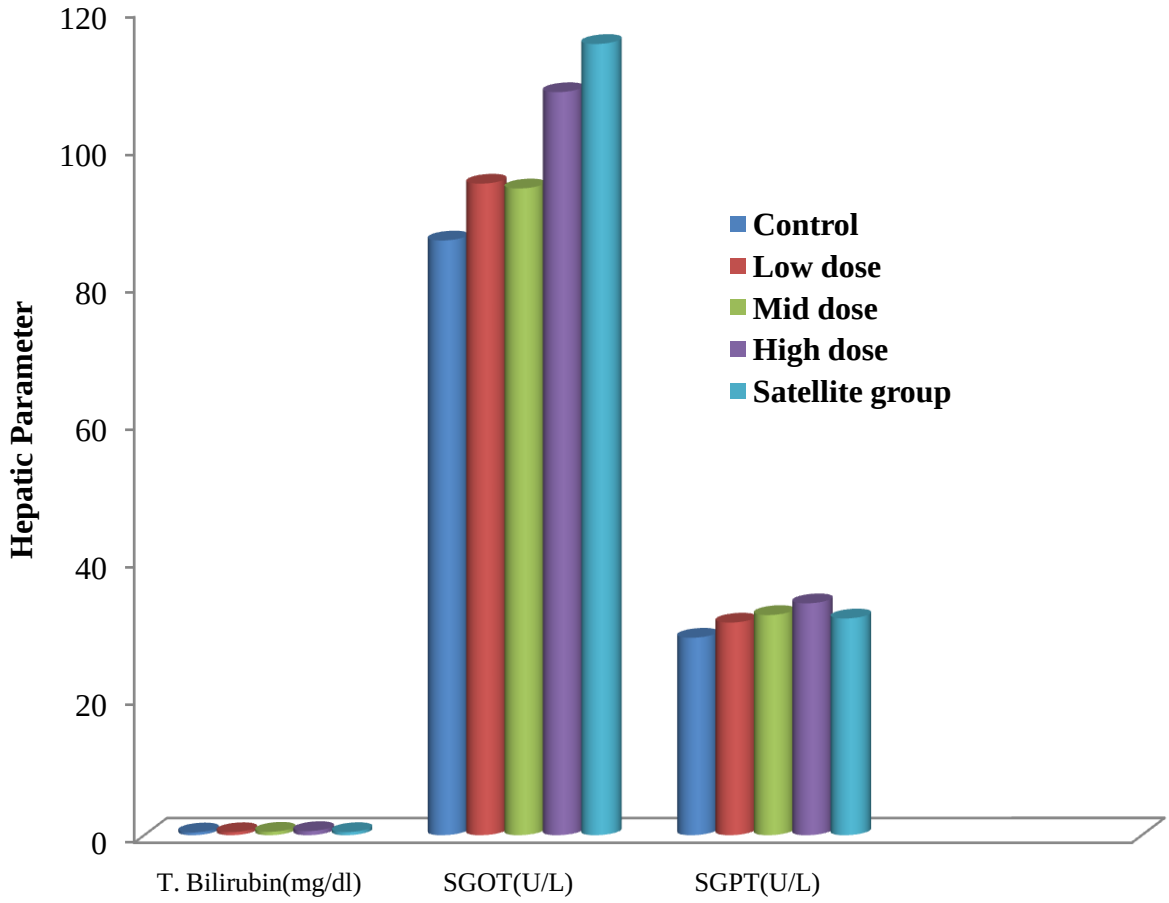
The results of the liver function test conducted at the end of the study the satellite group revealedsignificant changes in liver parameters when compared with control group.Other test group liver function parameters were normal when compared with control group.

Table 17, Effect of Ayapodi Elagam on Hepatic Parameters

Dose (mg/kg)	Control	Low dose	Mid dose	High dose	Satellite group
Total Bilirubin(mg/dl)	0.4±0.10	0.44±0.32	0.5±0.30	0.6±0.40	0.45±0.13
SGOT(U/L)	86.44±16.6	94.7±21.95	94±21.62	108.83±22.9	137±37.12**
SGPT(U/L)	28.7±7.8	30.9±5.7	32±9.2	33.7±5.5	31.5±7.6

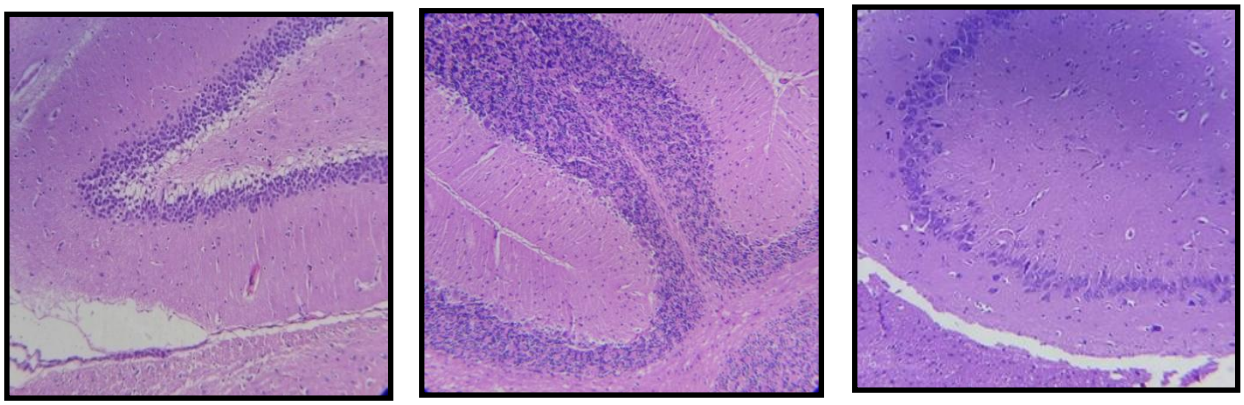
Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates that *P<0.05,**P<0.01

Figure 17: Effect of Ayapodi Elagam on Hepatic Parameters

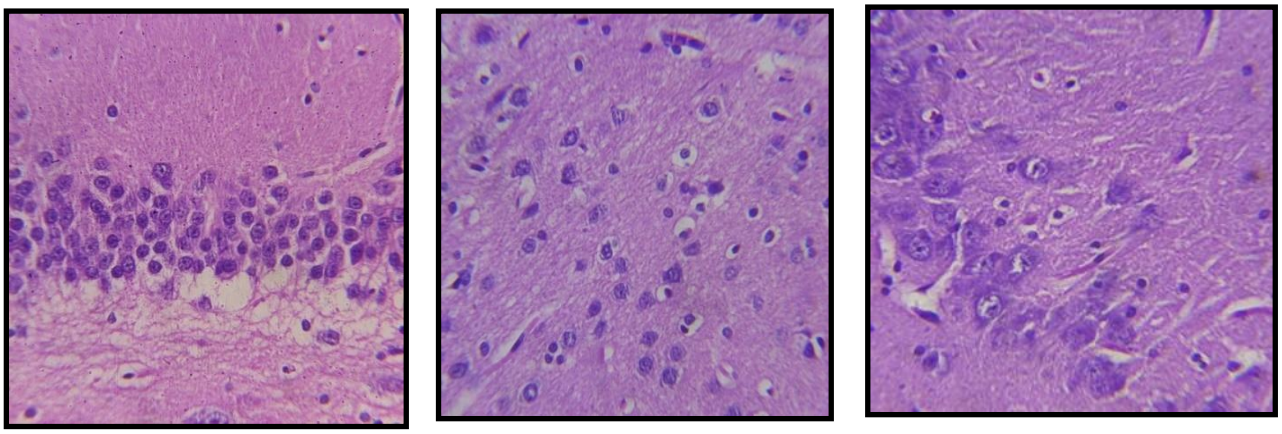


Histopathology of Brain (Male)

a) Control (10 X) b) High dose(10X) c) Satellite group (10X)



a) Control(40X) b) High dose (10X) c) Satellite group (10X)

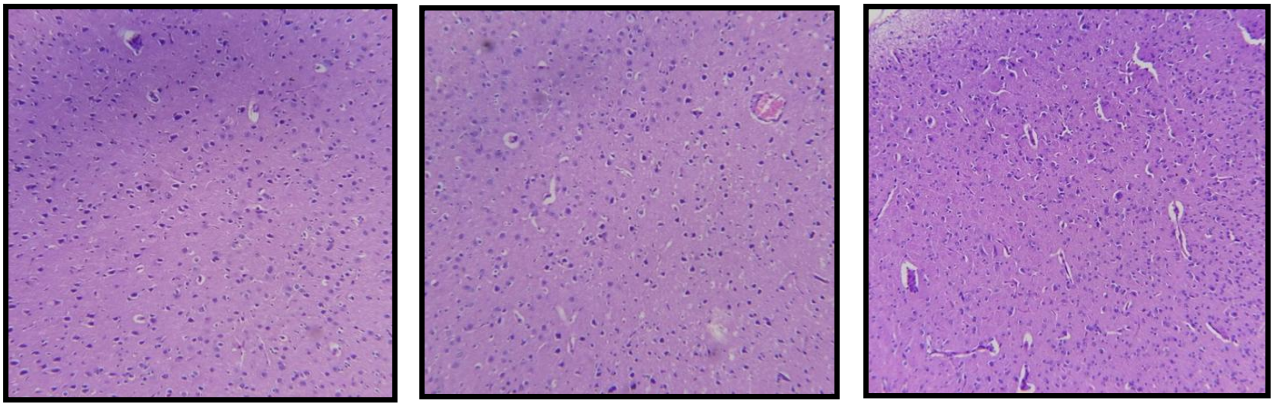


Report:

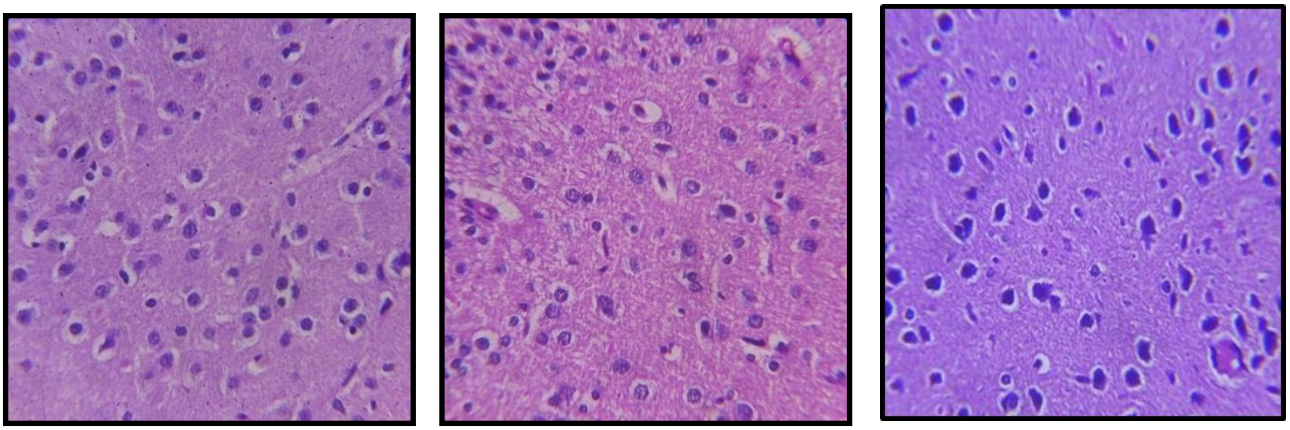
Appearance of Hippocampal neurons was normal with dense network.Morphology of neurons in CA1, CA2 and CA3 zones are normal.Cerebellum showed normal architecture in both cortex and medulla where three layers of cerebellar cortex the molecular, purkinje and granular layers appeared clear and distinct without any changes in their cells any inflammatory cells or gliosis.

Histopathology of Brain (Female)

a) Control (10X) b) High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

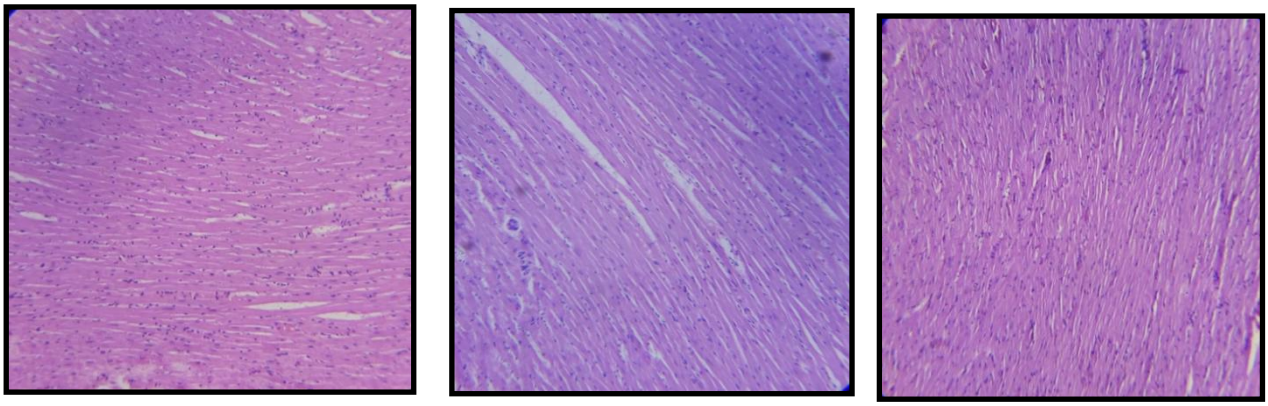


Report:

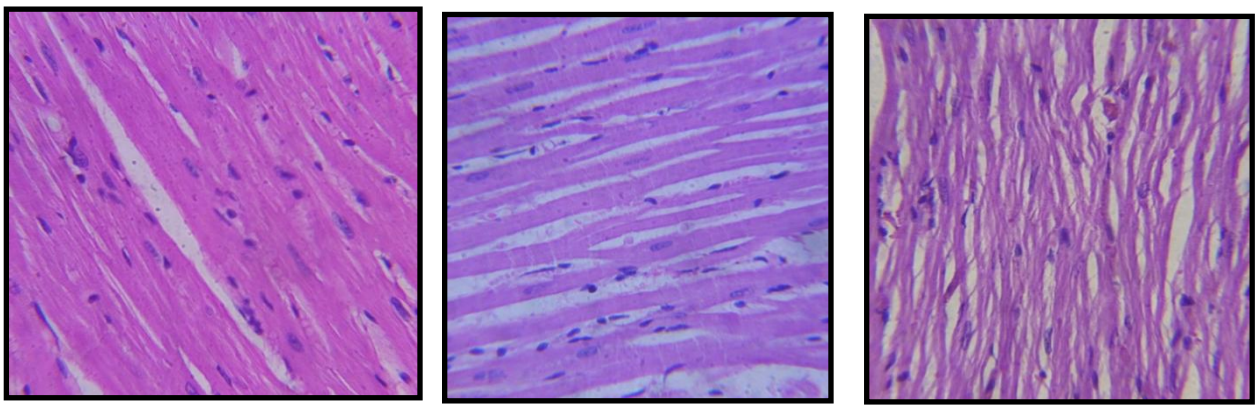
Regular marginal alignment on the neurons with promising histology was observed.No sign of vascular congestion and edema.Cerebral region shows the neuronal populations within the brain are heterogeneous with scattered combination of medium- to large-sized neurons with prominent nucleus.

Histopathology of Heart (Male)

a)Control (10X) b)High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satillite group(40X)

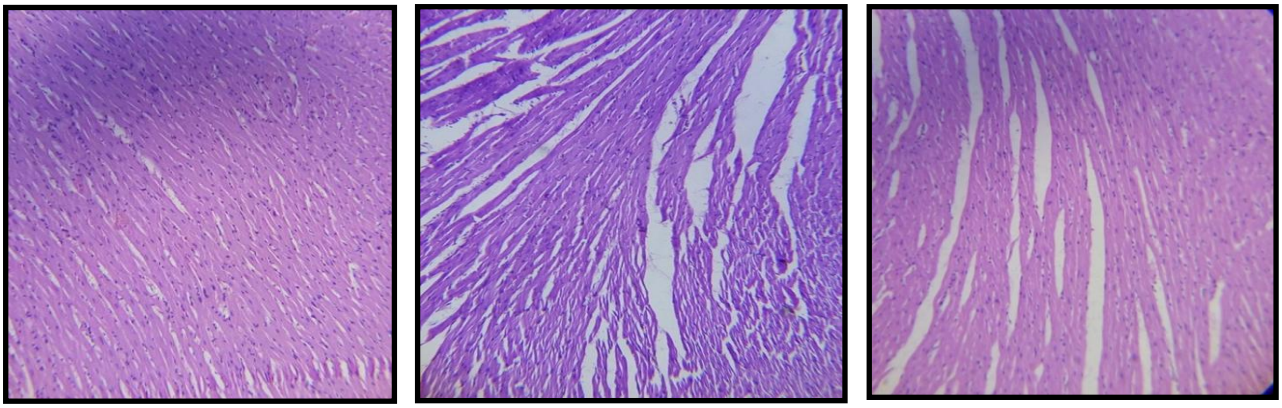


Report:

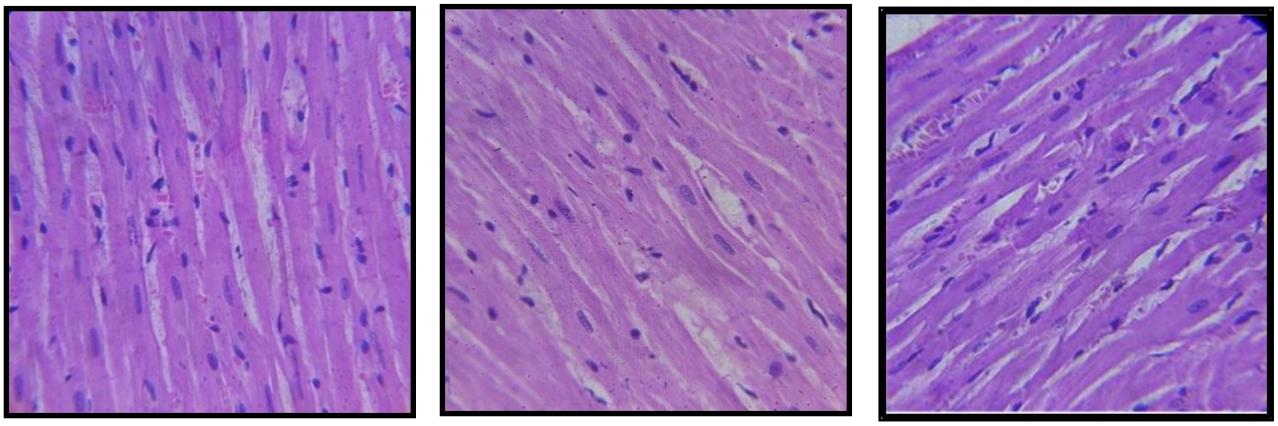
No evidence of necrotic myocardium . Appearance of fibrils and cross striations are equidistant.Fibres appears normal elongated and rod shaped.Myocardial cells appears normal with well-defined mycoplasma and prominent nucleus and nucleolus.Appearance of myocyte was normal.

Histopathology of Heart (Female)

a) Control (10X) b) High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

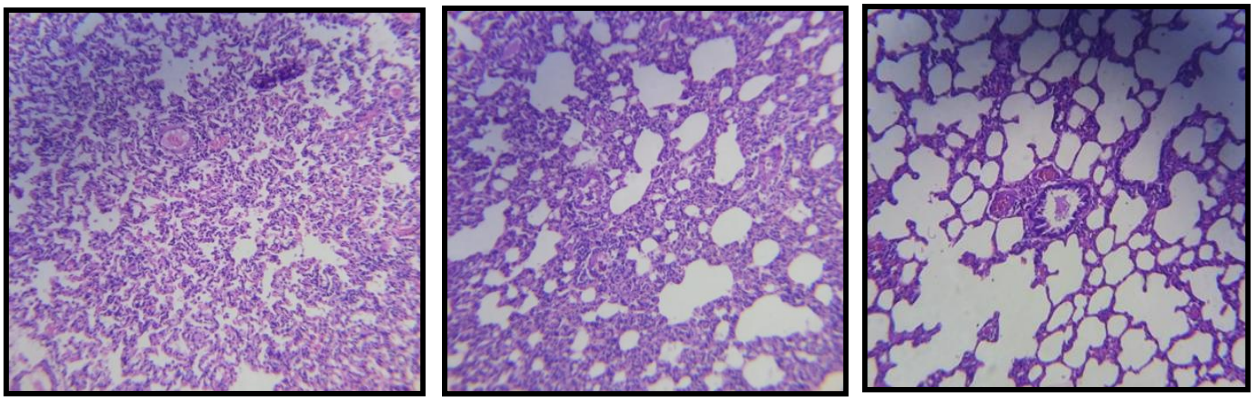


Report:

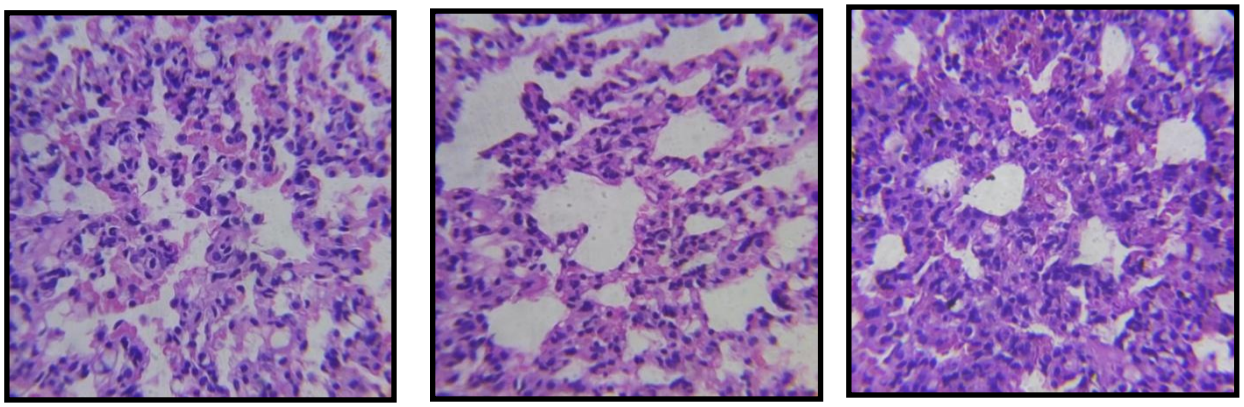
Nucleus appears prominent with regular arrangement of fibres. No evidence of pyknotic nucleus.No evidence of collagen deposition in myocardium.Appearance of myocyte was normal.Myocardial fibres appear normal elongated and rod shaped.

Histopathology of Lung (Male)

a) Control (10X) b) High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

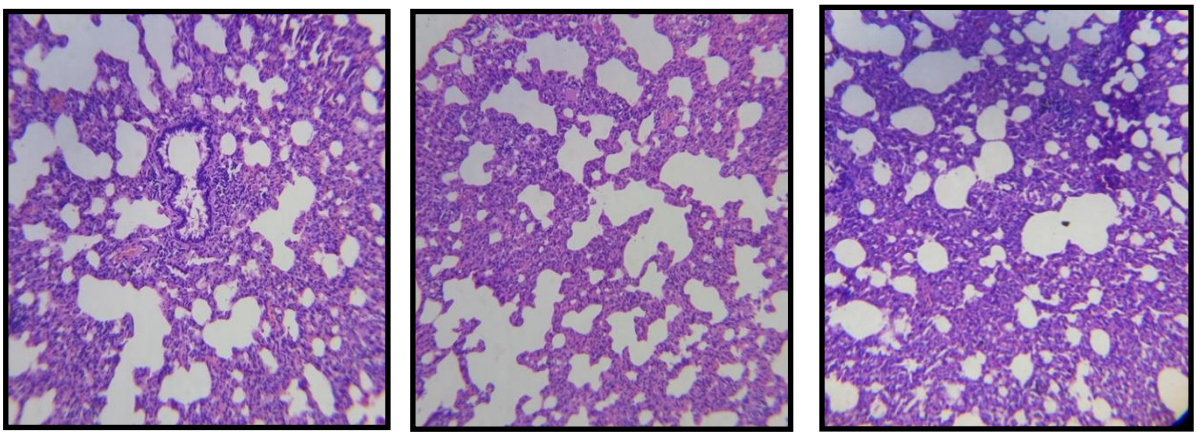


Report:

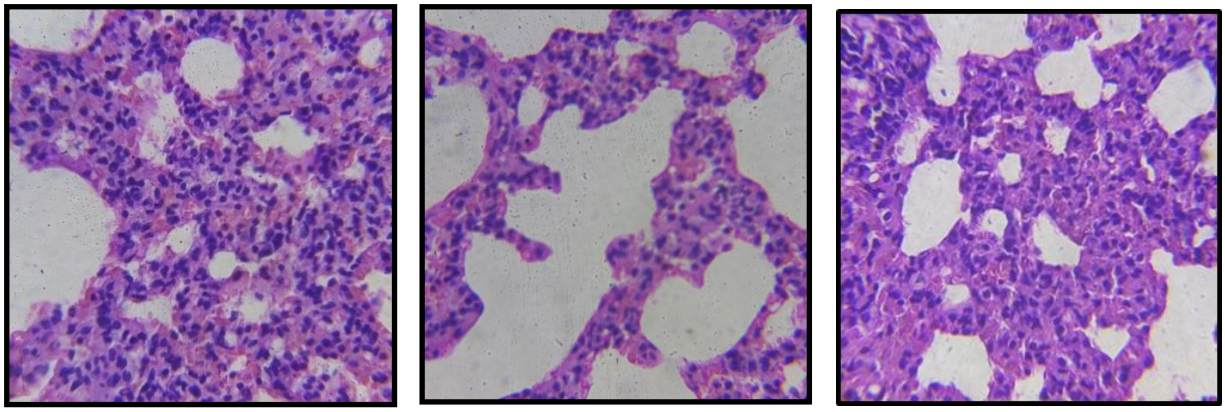
Perivascular region appears normal.Alveolar septa and wall appeared widen and normal.No signs of lymphocyte cuffing.Arrangement of epithelial and muscular appears normal.Opening of lumen of blood vessels appears regular with no invasion of inflammatory cells.Bronchial blood vessels and connective tissue appears normal with no sings of pulmonary edema.

Histopathology of Lung (Female)

a) Control (10X) b)High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

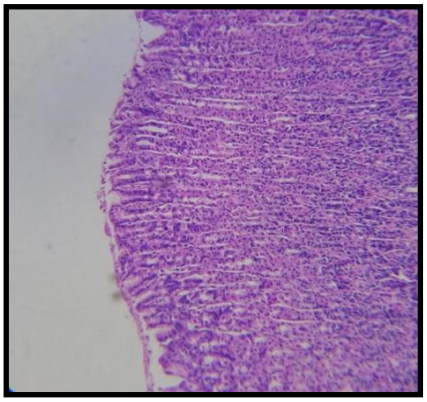
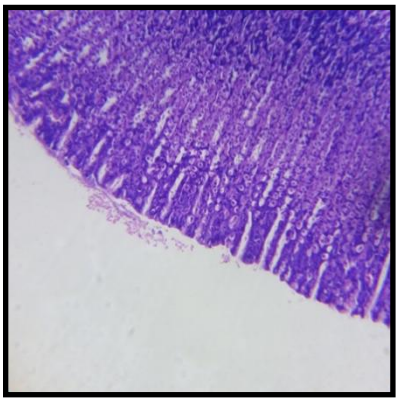
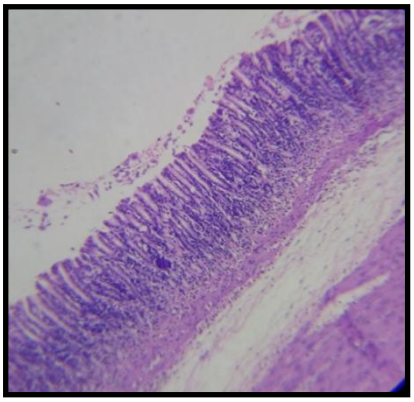


Report:

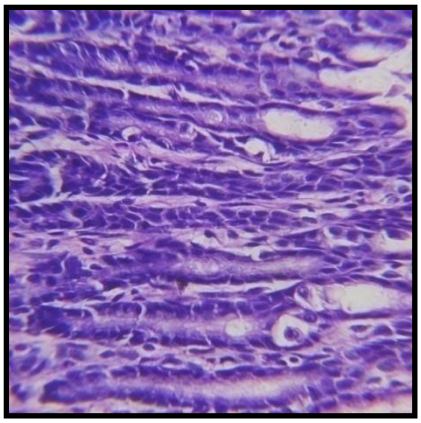
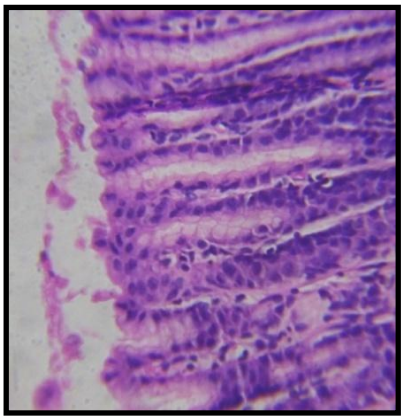
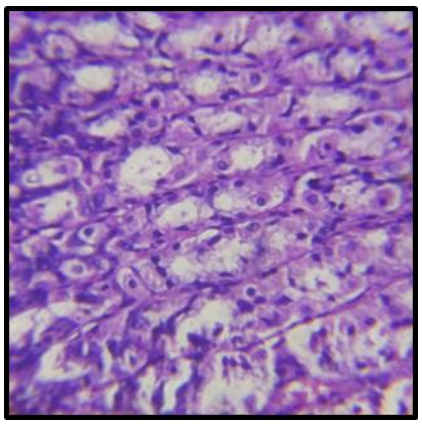
Bronchial opening appears regular with no signs of infiltration. Appearance of alveolar network was normal. The parenchyma appears normal with regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis.Nucleus of type I and II alveolar cells looks normal.

Histopathology of Stomach (Male)

a) Control (10X) b) High dose (10X) c) Satillite group(10X)

a) Control (40X) b) High dose (40X) c) Satellite group (40X)

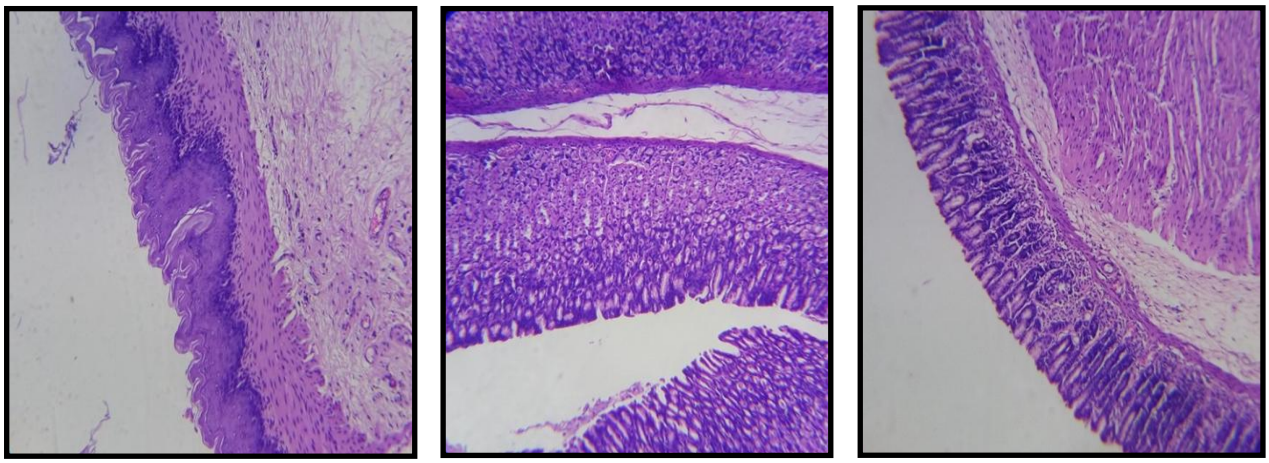




Report:

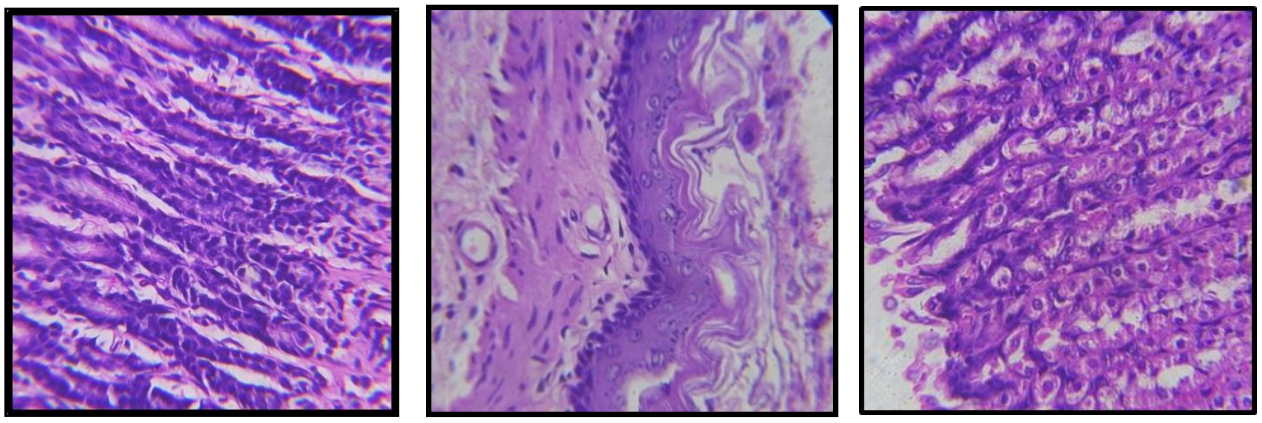
The continuity of mucosa was normal with no evidence of ulceration.Lamina propria appears normal with no evidence of infiltration and inflammation.Regular histology of Inner circular muscle (ICM), gastric pit (GP), and muscularis mucosae (MM) were observed.Gastric glands, gastric glands including secretary sheath appears normal.

Histopathology of Stomach (Female)

a) Control (10X) b)High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

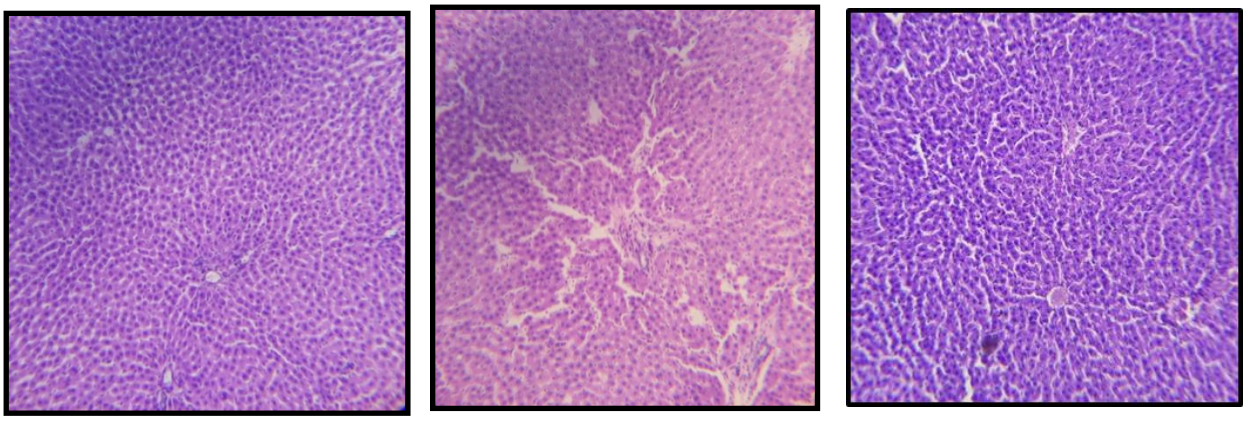


Report:

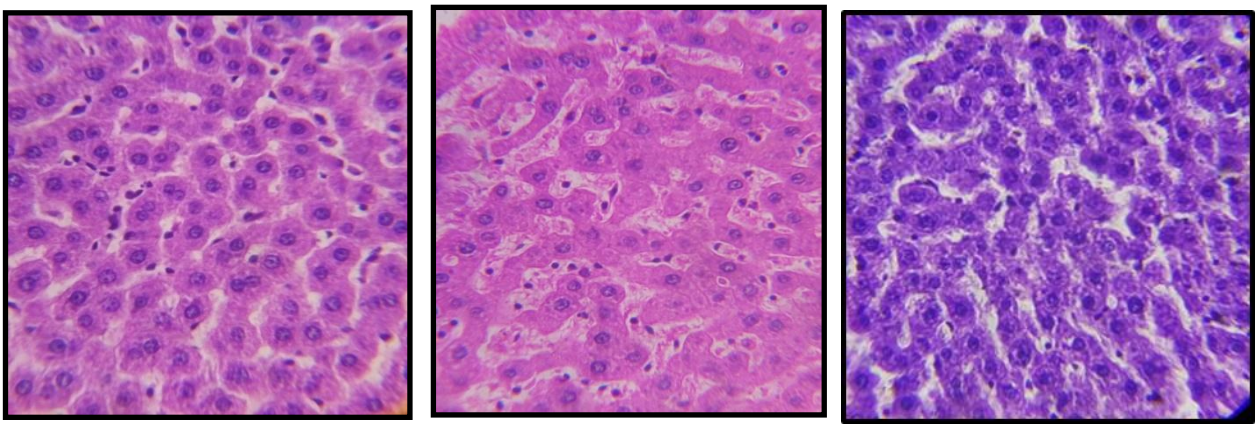
Mucosal wall appears normal with regular arrangement of connective tissue.No signs of ulcer and glandular degeneration were observed. Appearance of Sub-mucosa and gastric glands appear normal.Regular histology of Inner circular muscle (ICM), gastric pit (GP), and muscularis mucosae (MM) were observed.

Histopathology of Liver (Male)

a) Control (10X) b) High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

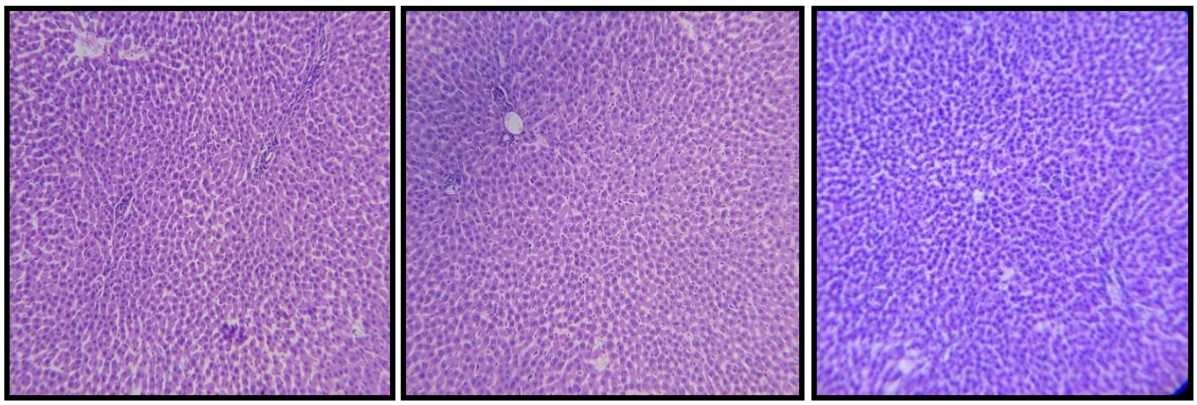


Report:

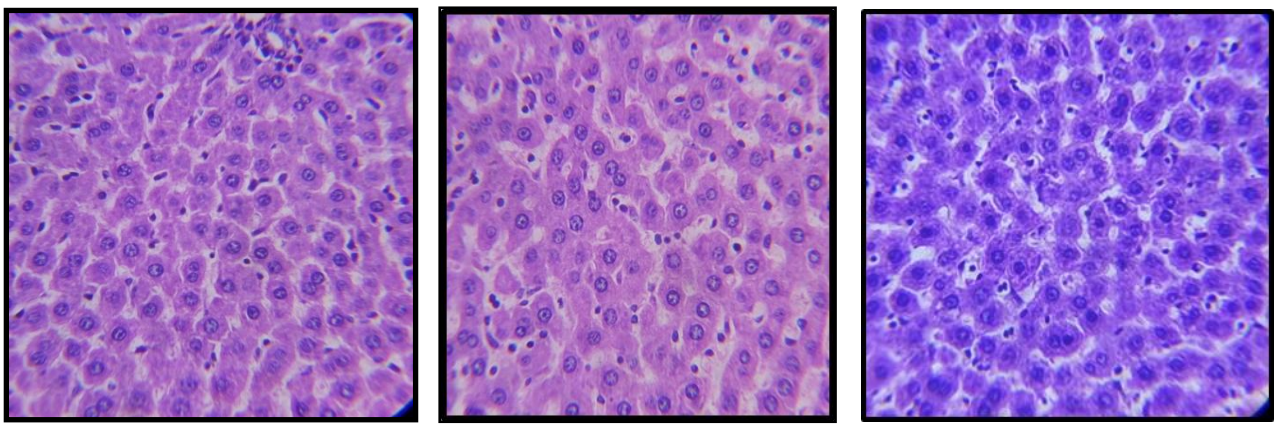
Cytoplasm appears normal with widen portal tract. Appearance of portal vein, bile duct and hepatic artery was normal.Mild discrete cytoplasmic vacuoles and rare foamy cytoplasm were observed.The sinusoid appears widen with occasional bi nucleated hepatocytes. Diffused vaculoar changes were observed in the mid zonal region.

Histopathology of Liver (Female)

a) Control (10X) b) High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

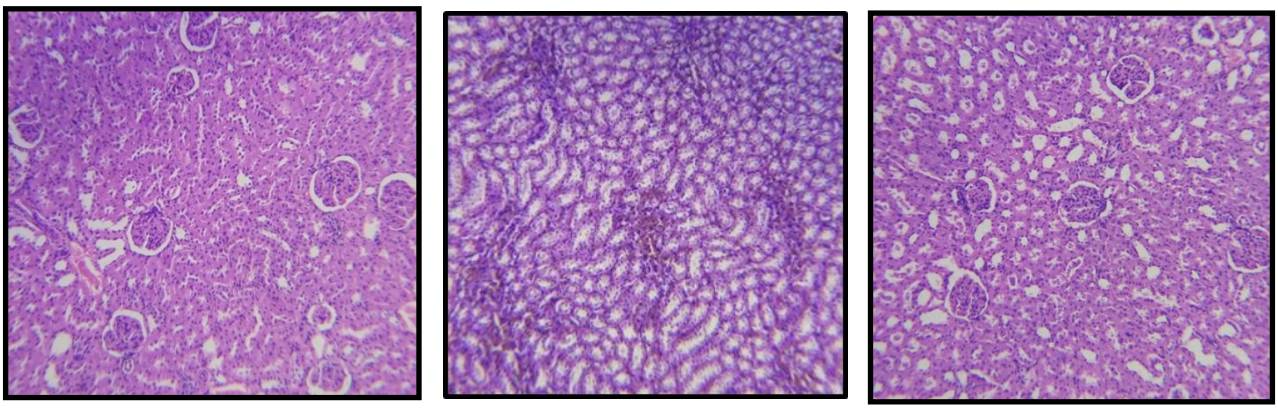


Report:

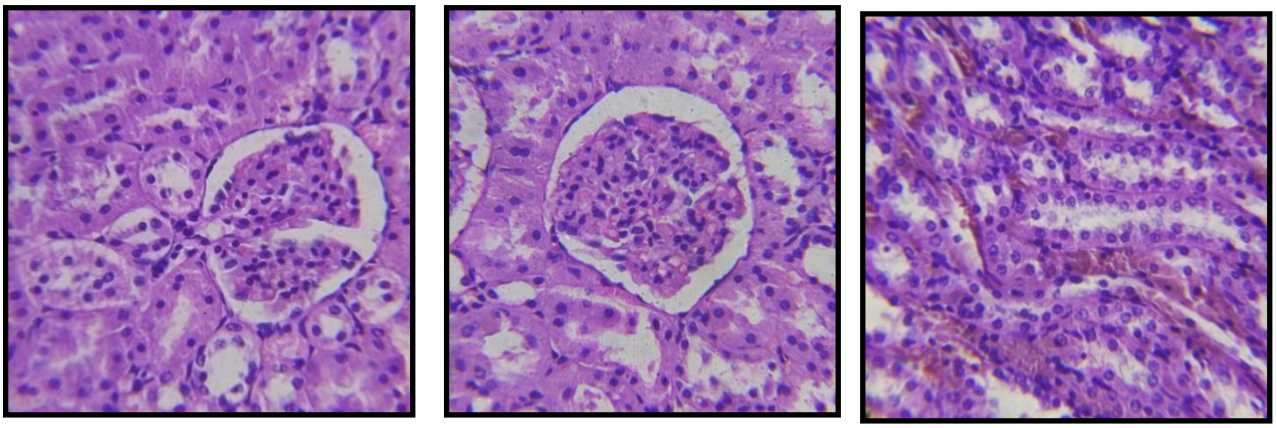
Appearance of portal vein, bile duct and hepatic artery was normal.Hepatocellular architecture, including hepatic sinusoid and hepatic cord was normal.The parenchyma appears normal with no evidence of necrosis. Appearance of terminal hepatic venules (central veins) to the portal tracts was normal.

Histopathology of Kidney (Male)

a) Control (10X) b) High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

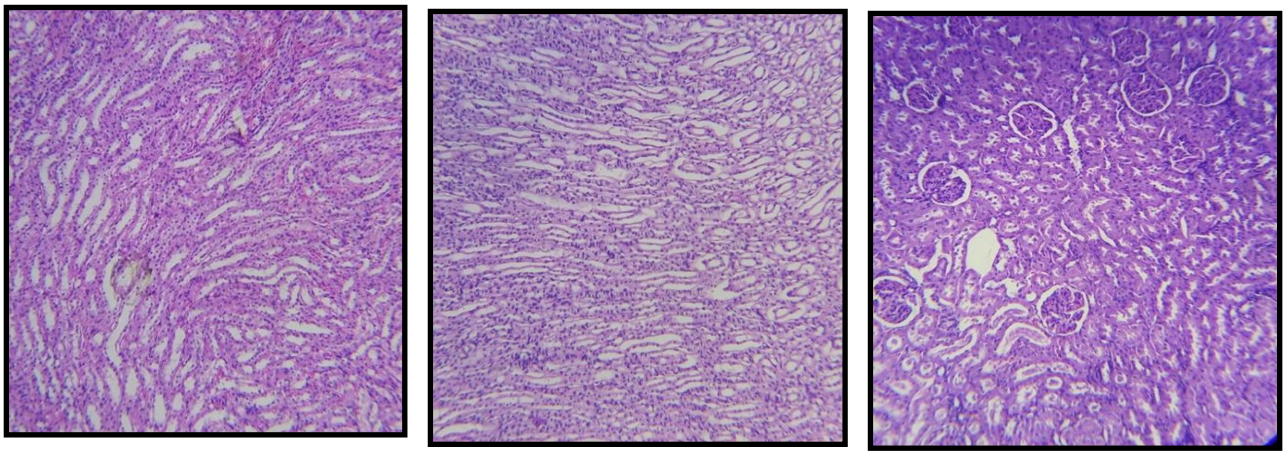


Report:

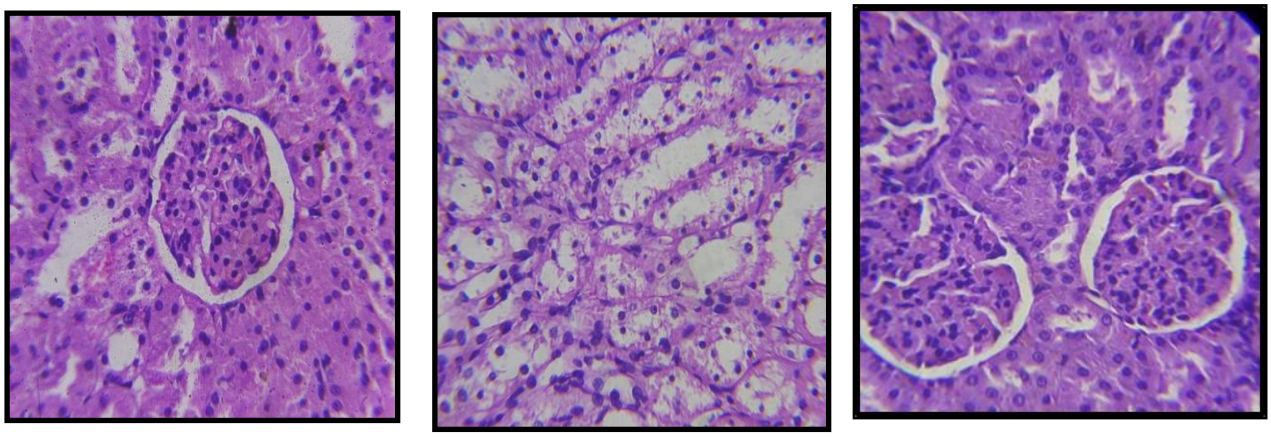
Lumen of vessels and bowman's space appears normal. Appearance of proximal and distal convolutes tubules was normal with no evidence of atrophy.The lining of epithelial cells in the renal tubules showed mild pyknosis of the nuclei.

Histopathology of Kidney (Female)

a) Control (10X) b) High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

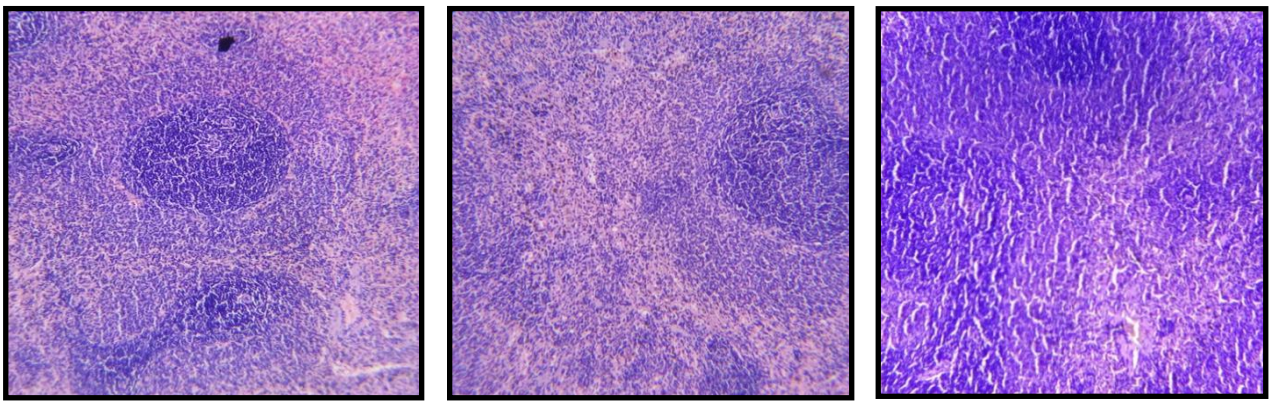


Report:

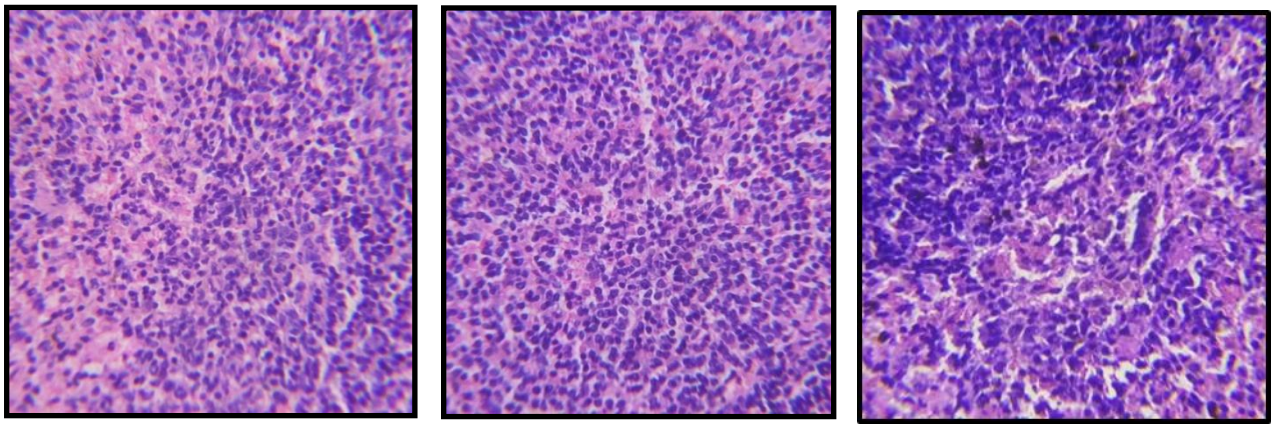
Normal renal structure with rounded renal corpuscles formed of the Glomerulus (G) and the Bowman's capsule (B) surrounded with Proximal Convoluted Tubule (PCT).Normal renal structure with regular arrangement of Distal Convoluted Tubule (DCT) and Collecting Duct (CD).

Histopathology of Spleen (Male)

a) Control (10X) b) High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

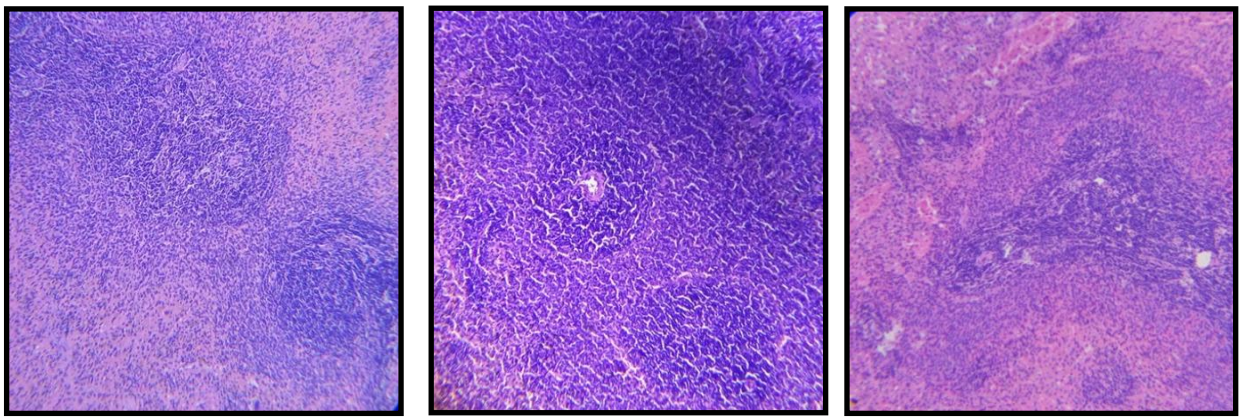


Report:

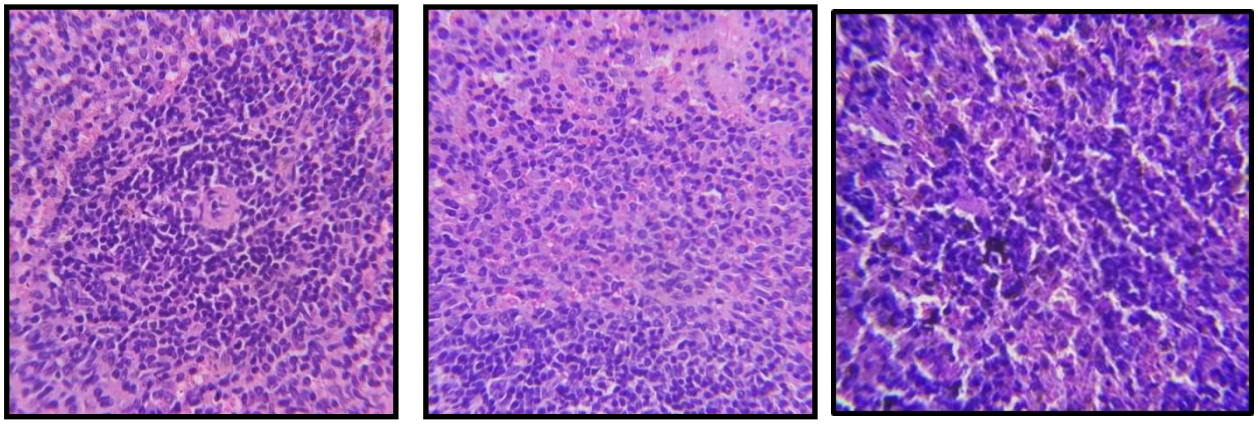
Erythropoietic cells (EP) are scattered throughout the red pulp of both the samples. No abnormalities found in lymph node of both the samples. Discrete changes were observe in spleen follicle and in pulp.

Histopathology of Spleen(Female)

a) Control (10X) b)High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

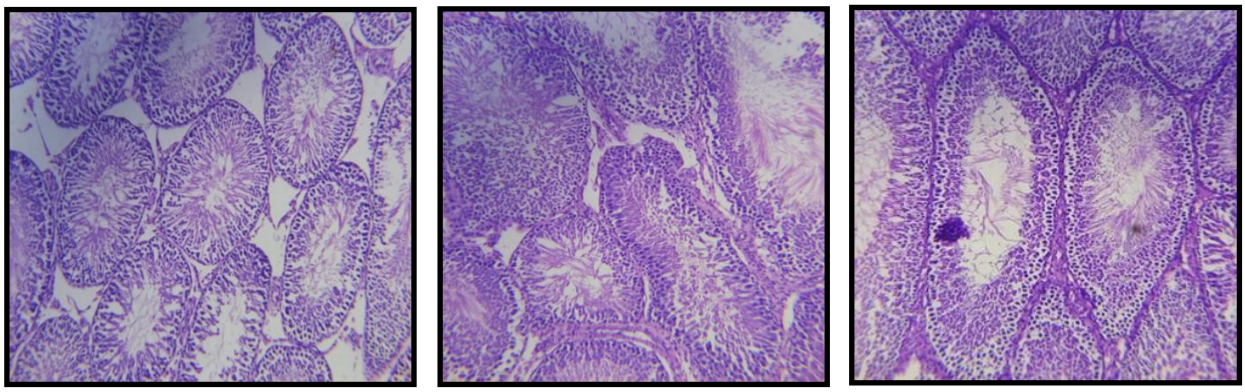


Report:

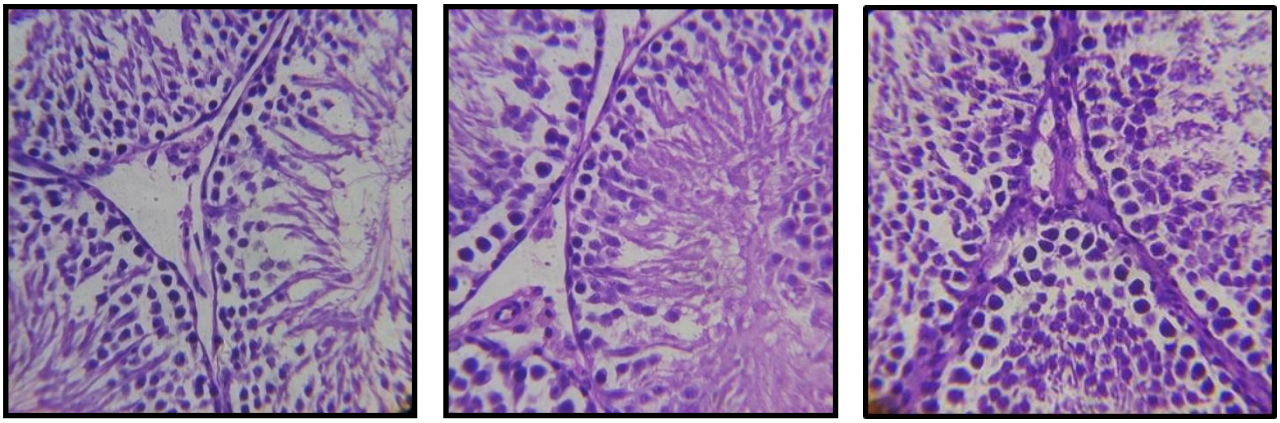
Appearance of LF – lymphoid follicle; PALS – periarterial lymphoid sheath was normal with no significant signs of enlargement. Remarkable changes were observed in spleen architecture with increased number of megakaryocytes. Appearance of splenic sinuses, Splenic cord and endothelial orientation was normal.

Histopathology of Testes

a) Control (10X) b) High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

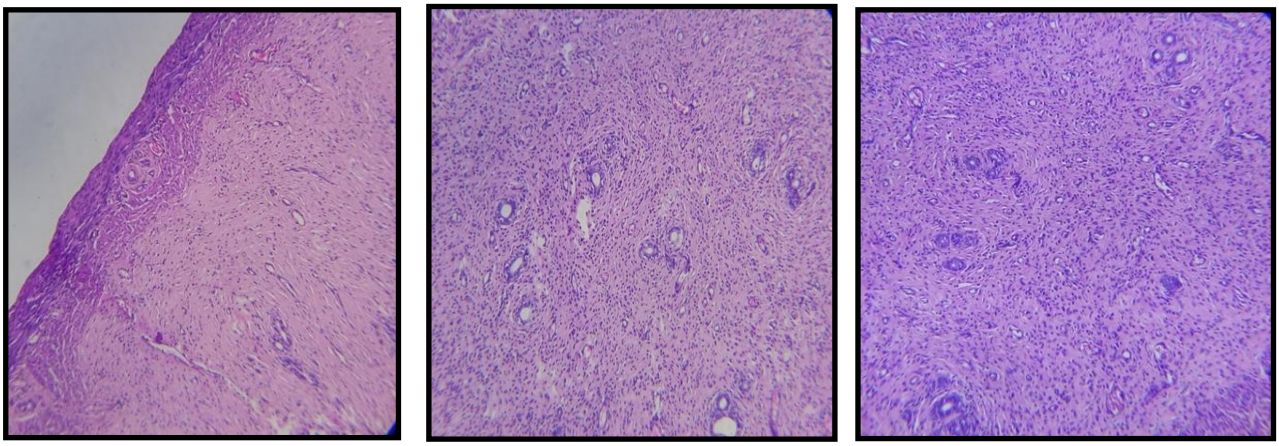


Report:

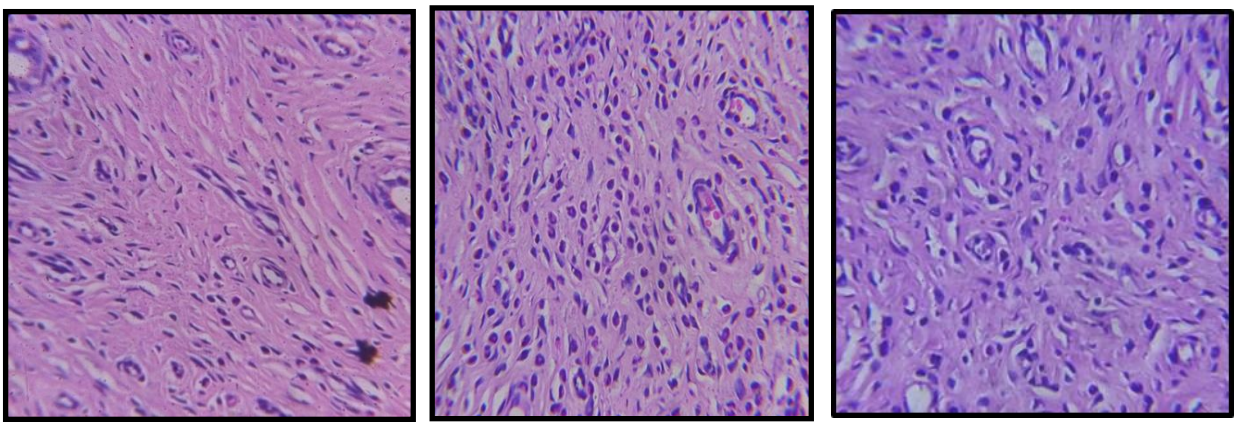
Light microscopic observation of the sample showing normal interstitial connective tissue with ovoid or polygonal leydig cells.Presence of mature somatic cells project the perfect histomorphology of testicular cells was observed. Appearance of leydig cells, interstitial tissue , seminiferous tubule, Sertoli cells and spermatogonia were normal

Histopathology of Uterus

a) Control (10X) b)High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)

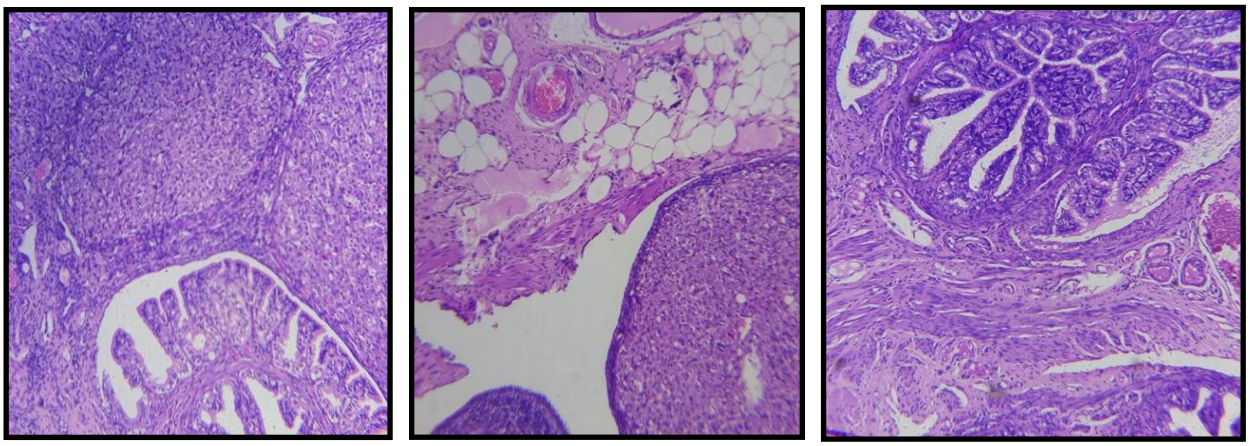


Report:

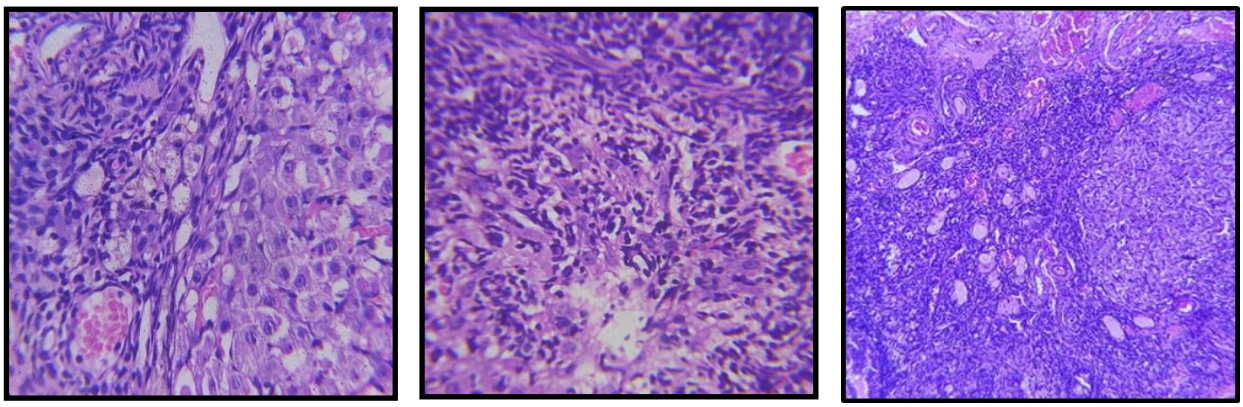
Endometrial stroma; G, gland; M, myometrium; P, perimetrium; L, lumen exhibits normal histological aspect of endometrium and myometrium.Appearance of uterine glands was normal. Endometrial gland, epithelium and blood vessels appears normal.

Histopathology of Ovary

a) Control (10X) b) High dose (10X) c) Satillite group(10X)



a) Control (40X) b) High dose (40X) c) Satellite group (40X)



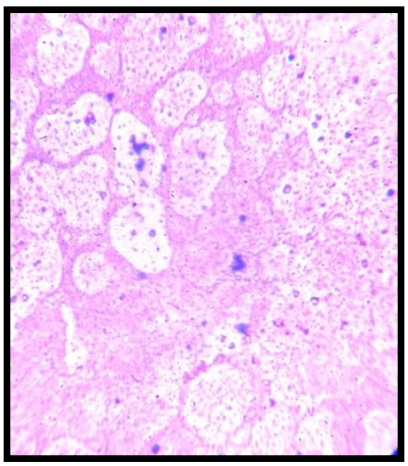
Report:

Sequential arrangement of granulosa cells arounds oocyte was normal and regular.Follicular cells, cytoplasm and nucleus appears normal.Histopathological analysis of ovary showed normal corpus luteum and Primordial follicles with few mature ovarian follicles with no signs of abnormality.

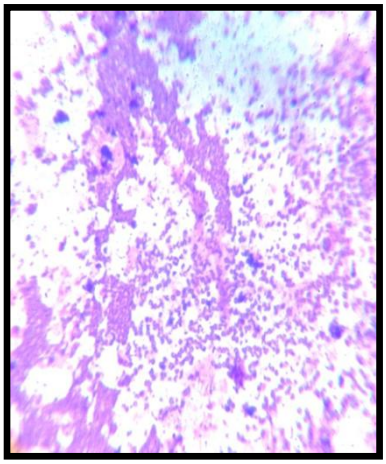
MICROSCOPIC VIEW OF BONE MARROW SMEAR OF RATS EXPOSED TO AYAPODI ELAGAM

LOW DOSE GROUP

Low Power Magnification (10X)

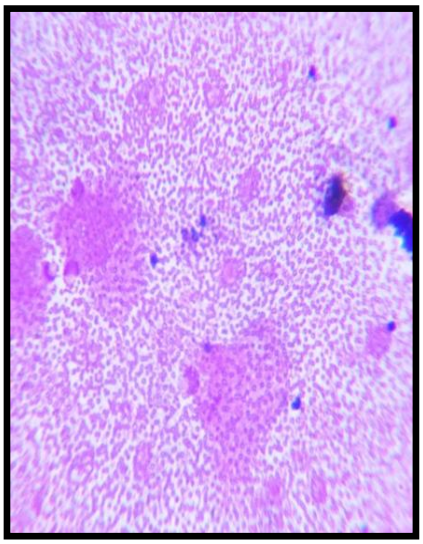


High Power Magnification (40X)

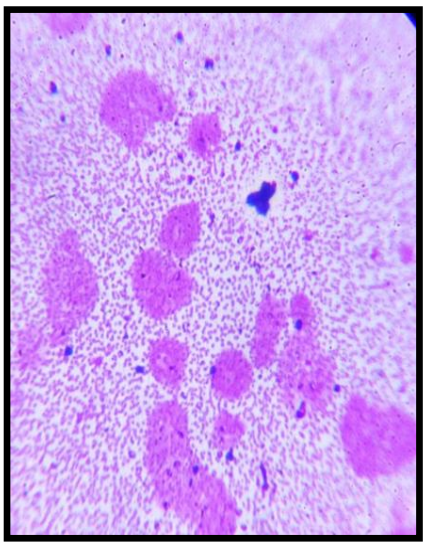


MID DOSE GROUP

Low Power Magnification (10X)

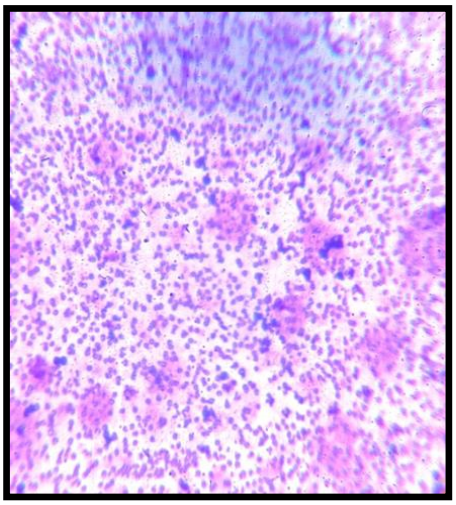


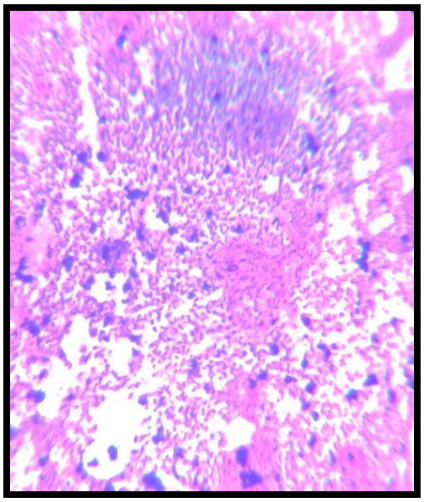
High Power Magnification (40X)



HIGH DOSE GROUP

Low Power Magnification (10X) High Power Magnification (40X)





SMEAR STUDY REPORT

Low Dose Group:

Bone marrow smear of Low dose reveals normal Eosinophilicmyelocyteand basophilicmyelocyte.

Mid Dose Group:

Increased network of erythroblastic islets were observed in mid dose.

High Dose Group:

Bulky prominent marrow with dense cellular portions were observed and active zones of erythropoiesis were observed in sample belong to HD.

DISCUSSION:

The Ayapodi Elagam is used for the treatment of PithaPandu, Pithavettai, and Kamalai etc., in Siddha system of medicine. One of the ingredients of this medicine was Iron (Ayam), has a long history in the treatment of anaemia among Siddha doctors in the Tamilnadu.

Literature reviews reveal that there was no such research has been done on Ayapodi Elagam. As an initial step in this present study a part of standardization of this drug

- AAS
- SEM
- EDAX
- Physiochemical
- Phytochemical analysis and Biochemical Analysis.

Its safety has been confirmed through acute and long-term oral toxicity study as per WHO guideline. Standardization of the drugs means confirmation of its identity and determination of its quality and purity.[88]By the way of standardization metal based drugs can be evaluated for their performances, limitation, optimal dosage, contraindications and applications.[89]

Physiochemical analysis of Ayapodi Elagam reveals that the drug appears black in colour and semisolid in nature. Its pH valves were acidic in nature which is expected to have significant absorption in the stomach.

The loss on drying at 105^0c was 5.23% hence it does not allow any microbial contamination in the drug. Extraction value determines the number of active constituents in a given amount of the formulation when extracted with a solvent media such as water and alcohol. The water-soluble and alcohol soluble extract values provide an indication of the extent of polar and non-polar compounds respectively present in Ayapodi Elagam. The extract values of Alcohol in Ayapodi Elagam is 14.1% and water is 37.18%.From the above result, we conclude that water is better solvent then alchohol.

Quality analysis of Ayapodi Elagam for acid radicals, basic radicals and other constituents demonstrates the presence of Ammonium, Magnesium, Aluminium, Calcium, Iron, Zinc, Phosphate, Carbonates, Reducing sugar, Alkaloids, Antipyrine, Aliphatic amino acids and Meconic acid are present. The presence of Zinc and Aminoacids helps to increase the iron absorption in the body. Phytochemical screening shows the presence of Carbohydrates, Glycosides, Flavonoids and Quinones. (From,the table 6).

The results of Atomic Absorption Spectrometric studies of Ayapodi Elagam for the determination of Iron prove that they are within the limit. The presence of Iron was found to be 12.94%.

Analysis of Ayapodi Elagam by Scanned Electron Microscopy revealed the size stabilization of particles on the process and the presence of micro size particles. The micro size particles can attach to the cell surface and can diffuse readily inside the cells. Thus, the size of the particle is able to influence the efficacy. The particles are irregular in shape with a smooth surface. The particles show the even distribution in the fields examined.

The FTIR spectrum shows theAyapodi Elagam sample and its functional groups. Before transfer, the bands from 3020 to 2952 cm−1 are ascribed to the stretching vibrations of the C–H bond of organic compounds. The band at 1140 cm−1 is assigned to the stretching vibration of the C–O bond of oleic acid. The band at 1423 cm−1 can be ascribed to the asymmetric and symmetric stretches of COO–, indicating that the acid chain is attached to the Fe_3O_4. The strong absorption band at 599 cm−1 is assigned to Fe–O vibrations of Fe_3O_4. There was a slight decrease in the bands intensity at 2510 cm−1, suggesting the reservation of N-H chains on the surface of Fe_3O_4. A band at 3437 cm−1 that corresponds to the vibration of O–H is enhanced due to absorbed water on the surface after transfer.This result concluded the function group of Ayapodi Elagam was Fe_3O_4.

EDAX analysis shows the elements present in the sample as shown in Fig. 2. The table represents the weight and atomic percentage of the sample. The presence of iron was 64 wt% because using magnetic separation during sample preparation. The presence of Si and Ca was less amount contributed by the presence of sand during sample collection and the herbal drugs Nellikai (*Phyllanthus Emblica*), Keezhkainelli (*Phyllanthusnirui*), Karisalai (*Eclipta alba*) in the sample.

In acute toxicity study carried out as per WHO guideline there was no treatment-related death or significance of toxicity developed in albino rats at dosage level 5000mg/kg/b.wtthroughout the study period.Behavioural pattern, food and water intake was normal. Further, no gross pathological changes have been seen in the internal organs of both control and treated groups. Thus the LD50 value was found to be greater than 5000mg/kg/b.wt.

To ensure the safety of Ayapodi Elagam long-term toxicity study was carried out as per WHO guideline. Except for hyperactivity at the time of drug administration, no other signs of toxicity and abnormal behavioural pattern were noted. After the blood collection, all the animals were euthanized for gross pathological examination of all major internal organs. The blood sample was sent to a lab for haematology and biochemical analysis. The organs were preserved in 10% buffered formalin solution before sending for histopathological study. All the reports were statistically analyzed.

Ayapodi Elagam shows significant reduction in the body weight of the low dose and mid dose group animals when compared with the control group.During the study period their behavioural pattern, food and water intake were normal within the physiological limit. And this study reveals that it does not adversely affect the basal metabolic process of the test group animals.

The hemopoietic system serves as an important target for the toxic chemicals and a sensitive index for pathological condition both in humans and animals. In haematological parameters, it had been observed that lymphocyte count was elevated after the administration of Ayapodi Elagam at the low dose and high dose group animals when compared to control group animals but the lymphocyte count was normal in post retrieval animals after one month of the drug with drawl.The other parameters such as Hb, RBC, WBC, DC, Platelet count, MCV, MCH and MCHC were normal in all the test group animals when compare to control group. The total cholesterol and LDL level were reduced in animal treated with the Ayapodi Elagam in the mid-dose group but they were in the normal limit. Transaminases (SGOT and SGPT) are good indicators of liver function and biomarkers to predict the possible toxicity of drugs. Any elevation pertaining to these enzymes indicate their outflow into the bloodstream due to damage in liver parenchymal cells. But, there was a marked increase in SGOT (Table – 17&Figure 15) in post retrieval group treated animals, when compared to control group, but it was also in the normal range

in other groups.In the present study there was no treatment-related abnormality in renal functions at all the test group animals (Table - 16& Figure14) and other hepatic parameters (Table 15& Figure13) were in normal limit.

The histopathological study the organ such as brain, heart, kidney, liver, lung, spleen, stomach, uterus, ovary, testis and bone marrow were taken. There is no pathological changes observed in high dose and satellite group animals when compare to control group of animal.

The smear study shows an increased network of erythroblastic islets was observed in mid-dose level.

Above the results indicates the Ayapodi Elagam treated animals were normal when it is administered at higher dose level (1800mg/kg).

SUMMARY:

The Ayapodi Elagam is used for the treatment of anaemia, jaundice etc., mentioned in the Siddha literature. One of the ingredient of the drug was iron .It has a long history in the treatment of anaemia. The literature review reveals that there was no such research has been done in Ayapodi Elagam. An initial step in this study a part of standardization and preclinical safety evaluation of this drug was done. The raw drug ayam was collected from the river bed and other herbal drugs were procured from farms. They were identified and authenticated. The Raw drugs were purified and the medicine was prepared as mentioned in the Siddha literature. On organoleptic examination, the finished product seems to be black in colour and semisolid in nature.

In the physicochemical analysis of Ayapodi Elagam reveals a loss on drying at 105^0C of 5.23% w/w. Total Ash value, Acid insoluble Ash value, Water, and Alcohol Soluble Extractive values reveal the purity of the test drug. Qualitative Analysis of Ayapodi Elagam demonstrates the presence of Ammonium, Magnesium, Aluminium, Calcium, Iron, Zinc, Phosphate, Carbonates, Reducing sugar, Alkaloids, Antipyrine, Aliphatic amino acids and Meconic acid.Phytochemical screening shows the presence of Carbohydrates, Glycosides, Flavonoids and Quinones. (From the table 6).The presence of Zinc and Aminoacids favour the iron uptake in the body.

The results of Atomic Absorption Spectrometric studies of Ayapodi Elagam showed the presence of Iron was found to be 12.94% in the drug.

Analysis of Ayapodi Elagam by Scanned Electron Microscopy revealed micro size particles and the particles are irregular in shape with a smooth surface.EDAX analysis shows the elements present in the sample was shown in Fig. 2. The table represents the weight and atomic percentage of the sample. The presence of iron was 64 wt %. The presence of Si and Ca was fewer amounts contributed.

The FTIR spectrum shows theAyapodi Elagamsample and its functional groups. Before transfer, the bands from 3020 to 2952 cm−1 are ascribed to the stretching vibrations of the C–H bond of organic compounds. The band at 1140 cm −1 is assigned to the

stretching vibration of the C–O bond of oleic acid. The band at 1423 cm−1 can be ascribed to the asymmetric and symmetric stretches of COO–, indicating that the acid chain is attached to the Fe_3O_4. The strong absorption band at 599 cm−1 is assigned to Fe–O vibrations of Fe_3O_4. This result concluded the function group of Ayapodi Elagam was Fe_3O_4.

In acute toxicity study carried out as per WHO guideline there was no treatment related death or significance of toxicity and abnormal behavioural patten was developed in albino rats at dosage level 5000mg/kg/b.wt throughout the study period. Further, no gross pathological changes have been seen in the internal organs of both control and treated groups. Thus the LD50 valve was found to be greater than 5000mg/kg/b.wt.

Long-Term Toxicity Study, there was no significant changes in behavioural pattern, food intake and water intake during the study period. The haematological parameter shows the elevated lymphocyte count seen in the low and high dose group animals but it becomes normal in post retrieval group animals. In the lipid profile the mid dose group animals shows significant reduction in Total cholesterol and LDL level it was normal in post retrieval group animals.

The histopathological study the organ such as brain, heart, kidney, liver, lung, spleen, stomach, uterus, ovary, testis and bone marrow were taken. There was no pathological changes observed in high dose, satellite group when compare to control the group of animals. The smear study shows an increased network of erythroblastic islets was observed in mid-dose level and high dose level.

The above studies explained the qualitative of drug and presence of Fe_3O_4 as afunctional group of the study drug. They microparticles were also excised in the test drug by studying various analytical methods. From the safety study it reveals that the low dose and mid dose treated animals had some minimal haematological changes, but post retrieval animals had normal physiological parameters. The histopathological chances were not observed .So the drug may be eliminated after completion of the treatment period of the animals. The smear study shows an increased network of erythroblastic islets. So the drug can increase the RBC production in anaemic patients. These findings were craved the safety uses of the metallic preparation used in Siddha system of medicines.

CHAPTER VIII

CONCLUSION:

Ayapodi Elagam had been used by Siddhars for a long time to treat various diseases such as Anaemia, Jaundice etc.From the study the drug was acidic in nature so it can be easily observed in the stomach.The presence of zinc and amino acids also fovour the iron absoption in the body. Acute toxicity study showed that the test drug can be used upto the dose level of 5000mg/kg/b.wt. Ayapodi Elagam does not produces notable abnormalities were observed in all the group of animal. Hence we conclude that the dosage of Ayapodi Elagam 2.5 – 5 gm twice a day mentioned in Anubogavaithiya Navaneetham is a safer therapeutic dose for the human. The smear study shows increase erythroblastic islets were observed.It also helps to increase Haemoglobin level of the anaemic patient.So the Ayapodi Elagam can be more effective and safety drug for the anemic patiens.The author hopes that this study will be a foot print for further research on pharmacological activity, teratogenicity and clinical trials regarding Ayapodi Elagam.

CHAPTER IX

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ANNEXURE

The following certificate are enclosed

- Research Methodology Certificate
- IAEC Certificate
- Authentication Certificate

The Tamil Nadu Dr.M.G.R.Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

This Certificate is awarded to Dr/~~Mr/Mrs~~.....A:...KALAIVANI...:.....

For participating as ~~Resource Person~~ / Delegate in the Twenty second Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

*For **AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 06th to 10th June 2016.

Dr.N.KABILAN, M.D.(S)
PROF & HEAD
DEPT.OF SIDDHA

Prof. **Dr.S.PUSHKALA,** M.D.,
REGISTRAR (FAC)

Prof. **Dr.S.GEETHALAKSHMI,** M.D., Ph.D.,
VICE CHANCELLOR

CERTIFICATE

This is certify that the project title....Pre clinical Safety.....
evaluation of "Ayapoodi Elagam" - 100 Rats (50 M+50 F)
Approval No: NIS/IAEC-III/2016
has been approved by the IAEC.

Prof. Dr. V. Balamurthi
Name of Chairman/~~Member Secretary~~ IAEC:

V. Balamurthi
Signature with date 23/09

Prof. Dr. K. Nachimuthu
Name of CPCSEA nominee:

28/9/2016

Chairman/~~Member Secretary~~ of IAEC:

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

NATIONAL INSTITUTE OF SIDDHA
MINISTRY OF AYUSH
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F.No:NIS/Gunapadam/Au/2017/2 18.03.17

AUTHENTIFICATION CERTIFICATE

Certified that the sample submitted for identification by **Dr. A. Kalaivani**, II year PG scholar, Dept. of Nanju noolum Maruthuva neethi noolum, National Institute of Siddha, Chennai - 47, voucher number 2, is identified as **Ayapodi- Iron Powder** on the basis of macroscopic character.

This certificate is issued for the purpose of preparing her dissertation medicine in Gunapadam laboratory, NIS.

Dr. S. Visweswaran, M.D (s)
Head of Department I/c
Department of Gunapadam
National Institute of Siddha
Tambaram Sanatorium, Chennai-47.

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs used in the formulation **Ayapodi Elagam** taken up for Post Graduation Dissertation studies by **Dr.A.Kalaivani** M.D.(S), II year, Department of Nanju Noolum Maruthuva Neethi Noolum, 2017, is identified and authenticated through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology, Micromorphology and Taxonomical methods as

Phyllanthus emblica Linn. (Euphorbiaceae), Fruit

Phyllanthus amarus Schum. & Thonn. (Euphorbiaceae), Whole plant

Eclipta alba Linn. (Asteraceae), Whole plant

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

Certificate No: NISMB2812017

Date: 06-03-2017

Authorized Signatory

Dr. D. ARAVIND, M.D.(s),M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha
Chennai - 600 047, INDIA

MADURAI KAMARAJ UNIVERSITY

(University with Potential for Excellence)

Palkalai Nagar, Madurai – 625 021

Two Day Workshop
on
NANOMATERIALS CHARACTERIZATION BY ELECTRON MICROSCOPY
(WNCEM- 2018)

21 - 22 February, 2018

Certificate

This is to certify that Dr./Mr./Ms. A. Kalaivani

From National Institute of Siddha

participated in the "Two Day Workshop on Nanomaterials Characterization by Electron Microscopy (WNCEM- 2018)" held during 21-22 February, 2018 at Madurai Kamaraj University, Madurai.

Convenor	Registrar	Vice-Chancellor
WNCEM-2018	Madurai Kamaraj University	Madurai Kamaraj University

INTRODUCTION

AIM AND OBJECTIVES

REVIEW OF LITERATURE

MATERIALS AND METHODS

PREPARATION OF THE TEST DRUG

QUALITATIVE ANALYSIS

SPECTROSCOPIC ANALYSIS

TOXICITY STUDIES

RESULTS

HISTOPATHOLOGY

SMEAR STUDY

DISCUSSION

SUMMARY

CONCLUSION

BIBLIOGRAPHY

ANNEXURE